Dissertation

on

"IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN-3 AND CD56 IN THYROID NEOPLASMS AND ITS HISTOPATHOLOGICAL CORRELATION"

Submitted in partial fulfillment of the regulations required for the award of

M.D. DEGREE

IN

PATHOLOGY – BRANCH-III

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

COIMBATORE MEDICAL COLLEGE

COIMBATORE

MAY 2019

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I hereby declare that the dissertation entitled **"IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN-3 AND CD56 IN THYROID NEOPLASMS AND ITS HISTOPATHOLOGICAL CORRELATION"** is a bonafide research work done by me in the Department of Pathology at Coimbatore Medical College during the period from January 2017 to June 2018 under the guidance and supervision of **Dr.C.LALITHA**, **M.D.**, Professor and Head of the Department, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai towards the partial fulfillment of the requirements for the award of M.D. Degree in Pathology (Branch - III). I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

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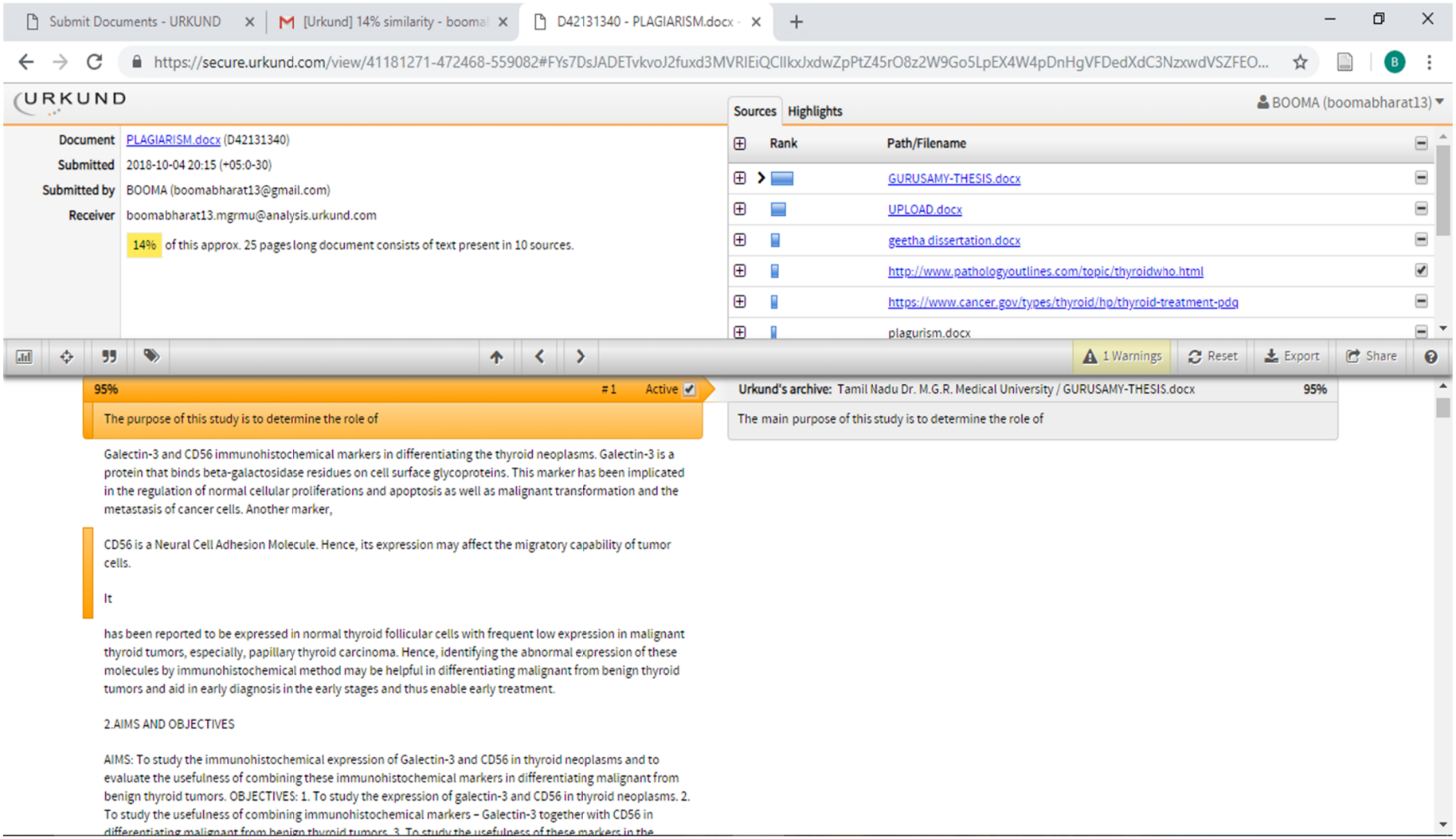
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The purpose of this study is to determine the role of
The main purpose of this study is to determine the role of
Galectin-3 and CD56 immunohistochemical markers in differentiating the thyroid neoplasms. Galectin-3 is a protein that binds beta-galactosidase residues on cell surface glycoproteins. This marker has been implicated in the regulation of normal cellular proliferations and apoptosis as well as malignant transformation and the metastasis of cancer cells. Another marker,
CD56 is a Neural Cell Adhesion Molecule. Hence, its expression may affect the migratory capability of tumor cells.
It
has been reported to be expressed in normal thyroid follicular cells with frequent low expression in malignant thyroid tumors, especially, papillary thyroid carcinoma. Hence, identifying the abnormal expression of these molecules by immunohistochemical method may be helpful in differentiating malignant from benign thyroid tumors and aid in early diagnosis in the early stages and thus enable early treatment.
2.AIMS AND OBJECTIVES
AIMS: To study the immunohistochemical expression of Galectin-3 and CD56 in thyroid neoplasms and to evaluate the usefulness of combining these immunohistochemical markers in differentiating malignant from benign thyroid tumors. OBJECTIVES: 1. To study the expression of galectin-3 and CD56 in thyroid neoplasms. 2. To study the usefulness of combining immunohistochemical markers – Galectin-3 together with CD56 in

CERTIFICATE OF PLAGIARISM

This is to certify that this dissertation work titled **IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN-3 AND CD56 IN THYROID NEOPLASMS AND ITS HISTOPATHOLOGICAL CORRELATION"** of the candidate **Dr.K.BOOMA** with registration number **201613252** for the award of **M.D. DEGREE** in the branch of **PATHOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains introduction to conclusion pages and result show **14%** percentage of plagiarism in the dissertation.

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1. INTRODUCTION

Thyroid carcinoma is the most common endocrine malignancy occurring worldwide and more than 95% of the thyroid carcinoma originate from follicular epithelial cells.[1] It has been found that 90% of the thyroid cancers have a favourable clinical outcome if treated at the earliest. There are four major subtypes of thyroid carcinoma of which papillary thyroid carcinoma constitutes more than 85% of all thyroid cancers; follicular carcinoma about 5-15%; anaplastic (undifferentiated) carcinoma less than 5% and medullary carcinoma about 5% of the cases. Papillary thyroid carcinoma is the most common thyroid malignancy. Classical form of papillary thyroid carcinoma does not pose a diagnostic difficulty. However, the follicular variant of papillary thyroid carcinoma poses a diagnostic challenge in which the differential diagnosis includes all other follicular patterned lesions like follicular adenoma and follicular carcinoma.

The purpose of this study is to determine the role of Galectin-3 and CD56 immunohistochemical markers in differentiating the thyroid neoplasms. Galectin-3 is a protein that binds beta-galactosidase residues on cell surface glycoproteins. This marker has been implicated in the regulation of normal cellular proliferations and apoptosis as well as malignant transformation and the metastasis of cancer cells.

Another marker, CD56 is a Neural Cell Adhesion Molecule. Hence, its expression may affect the migratory capability of tumor cells. It has been reported to be expressed in normal thyroid follicular cells with frequent low expression in malignant thyroid tumors, especially, papillary thyroid carcinoma.

Hence, identifying the abnormal expression of these molecules by immunohistochemical method may be helpful in differentiating malignant from benign thyroid tumors and aid in early diagnosis in the early stages and thus enable early treatment.

2. AIMS AND OBJECTIVES

AIMS:

To study the immunohistochemical expression of Galectin-3 and CD56 in thyroid neoplasms and to evaluate the usefulness of combining these immunohistochemical markers in differentiating malignant from benign thyroid tumors.

OBJECTIVES:

1. To study the expression of galectin-3 and CD56 in thyroid neoplasms.
2. To study the usefulness of combining immunohistochemical markers – Galectin-3 together with CD56 in differentiating malignant from benign thyroid tumors.
3. To study the usefulness of these markers in the diagnosis of thyroid neoplasms with equivocal morphologic features.

3. REVIEW OF LITERATURE

3.1) EMBRYOLOGY:

The thyroid gland is the first endocrine gland to appear in embryonic development, 24 days after fertilisation. It is of endodermal origin and begins as a diverticulum in foregut at the dorsum of tongue between tuberculum impar and hypobranchial eminence which corresponds to foramen caecum of adult tongue. The thyroid diverticulum descends caudally along the midline as thyroglossal duct in front of the hyoid bone and then divides into two parts, which later on develop as the two lobes of the thyroid.

Normally, the thyroglossal duct is obliterated and disappears - In 40% of individuals, it may form the pyramidal lobe.

3.2) ANATOMY:

The thyroid gland is butterfly shaped, situated in the lower part of the front and sides of the neck. It consists of two lobes – right and left which is bridged by isthmus and weighs about 15-25 grams and may have an ascending pyramidal lobe. The gland extends from C5 – T1, that is from middle of thyroid cartilage to 4^{th} or 5^{th} tracheal ring. Isthmus extends between 2^{nd} – 4^{th} tracheal ring. Each lobe measures approximately 5 x 2.5 x 2.5 cm and isthmus about 1.2 x 1.2 cm.

The gland is covered by thin fibrous capsule – outer and inner layer. Outer layer is continuous with pretracheal fascia, attaching the gland to the cricoid and thyroid cartilages, via a thickening of the fascia to form the posterior suspensory ligament of thyroid gland (Berry's ligament) – this is responsible for the movement of the gland with deglutition. Inner layer extends into the gland and divides it into lobules. Four parathyroid glands are situated two on each side, between the two layers of the capsule, posterior to the thyroid.

The thyroid gland is supplied by superior thyroid artery, branch of external carotid artery and inferior thyroid artery, branch of subclavian artery. The venous drainage is through superior and middle thyroid veins, which in turn drain into internal jugular vein and inferior thyroid vein respectively.

The lymphatic drainage is via prelaryngeal, pretracheal and paratracheal lymph nodes. The gland receives its nerve supply from **middle**, superior and inferior cervical ganglion of the sympathetic trunk and parasympathetic nerve supply from superior laryngeal nerve and recurrent laryngeal nerve.

3.3) HISTOLOGY:

The thyroid gland is divided into lobules comprising of 20-40 follicles, each lined by a single layer of cuboidal to low columnar epithelium. Follicles are the functional units. The lumen of the follicle is filled with colloid. Interfollicular spaces contain parafollicular cells - C cells, derived from neural crest.

3.4) PHYSIOLOGY:

Thyroid hormone is produced by three main steps :

1. Iodide uptake;
2. Iodide oxidation and organification and
3. Secretion of thyroid hormones.

There are 2 principal thyroid hormones produced – T4 (tetraiodothyronine / thyroxine) and T3 (triiodothyronine). They are responsible for regulation of basal metabolic rate, normal growth and development. T4 is produced in larger quantity but T3 is more potent than T4. These hormones are regulated by Thyrotropin Releasing Factor (TRF) from hypothalamus and Thyroid Stimulating Hormone (TSH) from pituitary. Parafollicular – C cells secrete calcitonin, essential for calcium metabolism.

3.5) THYROID NEOPLASMS:

GENERAL POINTS ON PRIMARY THYROID CANCERS:

1. Papillary carcinoma is the most common histologic type.[2]

2. Females are more commonly affected than men and are generally associated with a slightly better prognosis.[3]

3. Well differentiated tumors generally occur in younger patients, whereas in older patients less differentiated tumors are common.

4. Younger patients below 40 years of age generally have better prognosis than older patients.[4]

5. Primary tumor size and tumor staging are considered the most significant prognostic factors.[4]

DISTINCTIVE FEATURES OF THYROID CARCINOMAS IN CHILDREN:

1. Papillary carcinoma is the most common histologic type in children.

2. Irradiation is found to be an important factor for the development of thyroid cancer in children, for example, the Chernobyl nuclear accident.[5–9]

3. The cancer is commonly multifocal within the thyroid gland.

4. Lymph node metastasis is noted in 60-80% of children and the risk of recurrence is more common in these patients.[10]

5. Thyroid cancer in children is comparatively more aggressive than in adults, with more frequent extrathyroidal extension and higher incidence of lymph node or distant metastasis. However, children have a favourable prognosis with a mortality rate of 2.6% only.

3.6)WHO CLASSIFICATION OF THYROID TUMORS (2017)

1. Follicular adenoma.
2. Hyalinizing trabecular tumour.

3. Other encapsulated follicular patterned thyroid tumours:
    - Follicular tumours of uncertain malignant potential.
    - Noninvasive follicular thyroid neoplasm with papillary-like nuclear features.
4. Papillary thyroid carcinoma.
5. Follicular thyroid carcinoma (FTC), NOS:
    - FTC, minimally invasive.
    - FTC, encapsulated angioinvasive.
    - FTC, widely invasive.
6. Hurthle (oncocytic) cell tumours:
    - Hurthle cell adenoma.
    - Hurthle cell carcinoma.
7. Poorly differentiated thyroid carcinoma.
8. Anaplastic thyroid carcinoma.
9. Squamous cell carcinoma.
10. Medullary thyroid carcinoma.
11. Mixed medullary and follicular thyroid carcinoma.
12. Mucoepidermoid carcinoma.
13. Sclerosing mucoepidermoid carcinoma with eosinophilia.
14. Mucinous carcinoma.
15. Ectopic thymoma.

16. Spindle epithelial tumour with thymus-like differentiation.
17. Intrathyroid thymic carcinoma.
18. Paraganglioma and mesenchymal / stromal tumours:
    - Paraganglioma.
    - Peripheral nerve sheath tumours (PNSTs).
        - Schwannoma.
        - Malignant PNST.
    - Benign vascular tumours.
        - Haemangioma.
        - Cavernous haemangioma.
        - Lymphangioma.
    - Angiosarcoma.
    - Smooth muscle tumours.
        - Leiomyoma.
        - Leiomyosarcoma.
    - Solitary fibrous tumour.
19. Hematolymphoid tumours:
    - Langerhans cell histiocytosis.
    - Rosai-Dorfman disease.
    - Follicular dendritic cell sarcoma.
    - Primary thyroid lymphoma.

20. Germ cell tumours:

- Benign teratoma.
- Immature teratoma.
- Malignant teratoma.

21. Secondary tumours.

3.7) TNM STAGING OF THYROID TUMORS :

(AJCC CLASSIFICATION):

TUMOR (T):

Tx - Primary tumor cannot be assessed.

T0 - No evidence of primary tumor.

T1 - Tumor ≤ 2cm in greatest dimension, limited to the thyroid.

T1a - Tumor 1cm or less, limited to the thyroid.

T1b - Tumor >1cm but not >2cm in greatest dimension, limited to the thyroid.

T2 - Tumor > 2cm but ≤ 4cm in greatest dimension, limited to the thyroid.

T3 - Tumor > 4cm in greatest dimension limited to the thyroid or any tumor with minimal extrathyroidal extension

(e.g., extension to sternothyroid muscle or perithyroid soft tissue).

T4a - Moderately advanced disease.

Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus or recurrent laryngeal nerve.

T4b - Very advanced disease.

Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels.

All anaplastic carcinomas are considered under T4 tumors.

T4a - Intrathyroidal anaplastic carcinoma of any size.

T4b - Extrathyroidal anaplastic carcinoma of any size.

REGIONAL LYMPH NODES (N):

Nx - Regional lymph nodes cannot be assessed.

N0 - No regional lymph node metastasis.

N1 - Regional lymph node metastasis.

N1a - Metastasis to Level VI nodes (pretracheal, paratracheal, prelaryngeal or delphian nodes).

N1b - Metastasis to unilateral, bilateral or contralateral cervical (Levels I,II,III,IV or V) or retropharyngeal or superior mediastinal lymph nodes (Level VII).

DISTANT METASTASIS (M):

Mx - Distant metastasis cannot be assessed.

M0 - No distant metastasis.

M1 - Distant metastasis present.

STAGE GROUPING:

Based on histological type and age of the patient:

I. Papillary or follicular (less than 45 years):

➢ Stage I	Any T	Any N	M0
➢ Stage II	Any T	Any N	M1

II. Papillary or follicular (45years and above):

➢ Stage I	T1	N0	M0
➢ Stage II	T2	N0	M0
➢ Stage III	T3	N0	M0
➢ Stage III	T1	N1a	M0

- Stage III T2 N1a M0
- Stage III T3 N1a M0
- Stage IVA T4a N0 M0
- Stage IVA T4a N1a M0
- Stage IVA T1 N1b M0
- Stage IVA T2 N1b M0
- Stage IVA T3 N1b M0
- Stage IVA T4a N1b M0
- Stage IVB T4b Any N M0
- Stage IVC Any T Any N M1

III. **Medullary carcinoma:**

- Stage I T1 N0 M0
- Stage II T2 N0 M0
- Stage II T3 N0 M0
- Stage III T1 N1a M0
- Stage III T2 N1a M0
- Stage III T3 N1a M0
- Stage IVA T4a N0 M0
- Stage IVA T4a N1a M0
- Stage IVA T1 N1b M0
- Stage IVA T2 N1b M0
- Stage IVA T3 N1b M0

- Stage IVA T4a N1b M0
- Stage IVB T4b Any N M0
- Stage IVC Any T Any N M1

IV. Anaplastic carcinoma: (all cases are stage IV)

- Stage IVA T4a Any N M0
- Stage IVB T4b Any N M0
- Stage IVC Any T Any N M1

3.8) BENIGN THYROID TUMORS:

3.8.1) FOLLICULAR ADENOMA:

Follicular adenoma is the most common thyroid neoplasm. It is a benign encapsulated tumor showing follicular cell differentiation and lacks evidence of capsular, vascular or any other type of invasion and the nuclear features of the papillary family of neoplasms. Patients are mostly in euthyroid state and present with a lump in the thyroid. Usually, they are 'cold' nodules as they lack iodine uptake on radioactive iodine scans.

Patients with thyroid adenomas having elevated circulating levels of thyroglobulin,[11] associated with clinical hyperthyroidism and presenting as 'hot' nodules on iodine scans are called as toxic or PLUMMER ADENOMAS.[12,13] The thyroid outside hyperfunctioning nodules often contains intraluminal crystals of calcium oxalate, thought to be a sign of hypofunction.[14]

Gross:

Adenomas are almost always solitary. They are characteristically surrounded generally by a thin fibrous capsule. Size ranges from less than 1cm to about 10cm. Solid, fleshy, tan to brown tumor which may have secondary degenerative changes like hemorrhage, edema, fibrosis, calcification, bone formation and cystic degeneration.

Microscopy:

Encapsulated tumor.

Morphologic patterns:

1. Normofollicular (simple).
2. Macrofollicular (colloid).
3. Microfollicular (fetal) and
4. Trabecular/solid (embryonal).

Adenomas exhibiting papillary or pseudopapillary structures were previously referred as papillary adenomas are now termed as - Follicular adenoma with papillary architecture.

Differential diagnosis for adenomas with larger follicles:

1. Hyperplastic nodule.
2. Follicular variant of papillary carcinoma.

Histologic variants:

1. Hurthle cell adenoma.
2. Hyalinising trabecular adenoma.
3. Atypical adenoma – presents with irregular cytoarchitecture but lacks vascular or capsular invasion.[15]
4. Adenoma with bizarre nuclei.
5. Signet ring cell adenoma.

Other rare types:

6. With clear cell changes (including the signet ring, mucin-producing, and lipid-rich types).
7. Adenomas with adipose metaplasia of the stroma (so-called adenolipomas)[16]
8. Adenomas with cartilaginous metaplasia (so-called adenochondromas)[17]
9. Spindle cell adenomas (some vaguely resembling meningiomas),[18] and
10. Black adenomas – Minocycline induced massive deposition of cytoplasmic black pigment.[19]

Differential diagnosis for follicular adenoma:

1. Dominant nodule of nodular hyperplasia.
2. Minimally invasive follicular thyroid carcinoma and
3. Follicular variant of papillary thyroid carcinoma.

3.8.2) HURTHLE CELL ADENOMA:

Hurthle cells are also known as oncocytic, oxyphilic or Askanazy cells. They are cells with abundant brightly eosinophilic granular cytoplasm due to mitochondrial accumulation.

Hurthle cell adenoma is a variant of follicular neoplasm. Mostly adults, especially females are affected.

Gross:

Most are well encapsulated. Solid, tan to bright brown with areas of haemorrhage.

Microscopy:

Cells are arranged in follicular, trabeculae, solid sheets or papillary pattern. The inspissated intraluminal colloid may be calcified, mimicking psammoma bodies. Individual cells have abundant brightly eosinophilic granular cytoplasm and round nuclei with granular to coarse chromatin and

distinct nucleoli. Occasional nuclear grooves or nuclear pseudo-inclusions can be present.

Features in favour of malignancy in Hurthle cell adenoma:

- Presence of capsular or vascular invasion.[20–24]
- Tumor size $\geq$ 4cm.[25]

Old age, larger tumor size (>4cm) and extensive vascular invasion are factors associated with worse prognosis.[26,27]

3.8.3) HYALINISING TRABECULAR ADENOMA:

They are peculiar type of benign follicular cell neoplasm – 1st identified by Langhans.[28] Also known as Paraganglioma – Like Adenoma of Thyroid (PLAT). Females are more commonly affected.

Composed of cells arranged in wavy and coiled trabeculae, interspersed with occasional small cystic spaces. Individual cells are elongated to polygonal with lightly eosinophilic cytoplasm. The elongated tumor cells are aligned perpendicular to the trabeculae. Hyaline material is seen in both the cytoplasm of tumor cells and in the extracellular space. Nuclear grooves, pseudoinclusions and perinucleolar haloes are also seen.

Cytoplasmic yellow body - a round, pale yellow inclusion body in the paranuclear region of the cytoplasm, refractile in nature and detectable both in tissue sections and in fine needle aspiration smears[29] is considered a distinctive

(not specific) feature of hyalinising trabecular adenoma. This structure represents giant lysosomes ultrastructurally.[30]

IMMUNOHISTOCHEMISTRY:

TTF-1 is expressed by most tumor cells. Hyalinizing trabecular adenoma frequently shows a unique pattern of cytoplasmic granular and cell membrane staining with MIB-1 antibody, but not with the polyclonal Ki-67 antibody.[31,32] Galectin-3 expression is seen in about half of the cases[33] and is much lower in hyalinising trabecular adenoma than in papillary thyroid carcinoma.[34] Shows focal and inconstant reactivity for neuroendocrine markers such as NSE and neurotensin.[35] HBME-1 is consistently negative in hyalinising trabecular adenoma.[36]

Differential diagnosis for Hyalinising trabecular adenoma:

1. Papillary carcinoma of thyroid.[37]
2. Paraganglioma.
3. Medullary carcinoma of thyroid.

3.9) MALIGNANT THYROID TUMORS:

3.9.1) PAPILLARY CARCINOMA:

Papillary carcinoma is the most common differentiated malignant neoplasm of the thyroid.[38] It constitutes about 80% of all thyroid carcinomas. The peak age of incidence is between 30-40 years. Females are more commonly

affected. It accounts for more than 90% thyroid malignancies in children. It spreads by local invasion and to the lymph node. Cervical lymph node is more commonly involved, especially in young patients. Distant metastasis is uncommon.

Risk factors:

1. Ionising radiation exposure.
2. Genetic factors.
3. Hashimoto thyroiditis.

Gross:

-Solid to variegated, tan to white, firm in consistency.

- Usually infiltrative with irregular ill-defined borders.

- May undergo cystic changes, fibrosis and calcification.

- Papillary formations may be sometimes seen grossly.

Microscopy:

Characterised by the presence of complex branching papillae as finger like processes, with central fibrovascular core. The papillae are lined by cuboidal cells with the nuclear features typical and characteristic of papillary carcinoma which includes nuclear crowding and overlapping,[39] ovoid nuclei with finely dispersed pale to clear chromatin and peripheral condensation of the cytoplasm (Ground glass appearance, **Orphan Annie Eye** nuclei), irregular

nuclear membrane, eosinophilic intranuclear cytoplasmic inclusions and nuclear longitudinal grooves.[40,41]

The stroma between tumor cells may show psammoma bodies in 50% of cases with papillary carcinoma. They are basophilic lamellated calcific spherules resembling inspissated colloid. They are typical but not diagnostic.

Morphologic variants of papillary thyroid carcinoma:

- ❖ Conventional.
- ❖ Columnar cell.
- ❖ Clear cell.
- ❖ Cribriform morular.
- ❖ Diffuse sclerosing.
- ❖ Follicular.
- ❖ Macrofollicular.
- ❖ Microcarcinoma.
- ❖ Oncocytic or oxyphil cell.
- ❖ Solid.
- ❖ Tall cell.
- ❖ PTC with prominent hobnail features.
- ❖ PTC with nodular fasciitis like stroma.

COLUMNAR CELL VARIANT:

This is a rare and more aggressive variant of thyroid neoplasm. It is characterized by mixed papillary, complex glandular, cribriform and solid patterns. The papillae and glands are lined by tall columnar cells with pseudostratified hyperchromatic oval or elongated nuclei. They lack the typical nuclear features of papillary thyroid carcinoma. Extrathyroidal extension, distant metastases (especially to lung and vertebra) and regional lymph node metastases are common.

CLEAR CELL VARIANT:

This is an uncommon variant with papillary or follicular pattern of cells, having clear vacuolated cytoplasm due to accumulation of glycogen. They have the typical nuclear features of papillary thyroid carcinoma.

CRIBRIFORM MORULAR VARIANT:

This is a rare variant associated with Familial Adenomatous Polyposis syndrome. It is characterized by a prominent cribriform pattern, with interspersed squamoid islands (morules) that contain cells with homogenous, lightly eosinophilic, biotin-containing inclusions in the nuclei. Characteristically, the luminal spaces of follicles are devoid of colloid. Some nuclei exhibit nuclear groove.

DIFFUSE SCLEROSING VARIANT:

Children and adolescents are more commonly affected. This variant is considered more aggressive than conventional papillary carcinoma, due to its higher incidence of extrathyroidal extension, lymph node metastasis (nearly 100%) and distant metastasis.[42–47]

Typical histologic features:[48–50]

1. Diffuse involvement of one or both lobes.
2. Dense sclerosis.
3. Heavy lymphoplasmacytic infiltrate.
4. Abundant psammoma bodies.
5. Scattered small islands of papillary carcinoma with prominent squamous or squamoid differentiation.
6. Extensive lymphatic permeation.[51]

FOLLICULAR VARIANT:

This variant of papillary thyroid carcinoma is composed entirely of follicles[52] and is aggressive in nature due to its ability to metastasize distally.

Histologic features:

1. Infiltrative type of growth.
2. The follicles are elongated and vary in size and shape.

3. The colloid is typically deeply eosinophilic with scalloped edges.[53]
4. Presence of abortive papillae.
5. Typical nuclear features of papillary thyroid carcinoma.
6. Psammoma bodies.
7. Sclerosis may be present.

Encapsulated follicular variant of papillary thyroid carcinoma is termed as LINDSAY TUMOR and is surrounded by a fibrous capsule.

MACROFOLLICULAR VARIANT:

This variant is composed of large dilated colloid filled follicles of varying sizes. The cells lining the macrofollicles may not display the characteristic nuclear features of papillary carcinoma. Nuclei lining the follicles may be hyperchromatic.

Differential diagnosis:

1. Nodular goitre.
2. Macrofollicular adenoma.

MICROCARCINOMA:

Papillary carcinoma measuring 1 cm or less in diameter are termed as papillary microcarcinoma. Previously known as occult sclerosing carcinoma or nonencapsulated sclerosing tumor.[54] More common in males than females.[55] It is an incidental finding but has an excellent prognosis.

ONCOCYTIC or OXYPHILIC VARIANT:

May be encapsulated or invasive. Composed predominantly of cells containing abundant eosinophilic granular cytoplasm due to accumulation of mitochondria.[56] Typical nuclear features of papillary thyroid carcinoma are seen.

SOLID VARIANT:

Common in children. Composed of solid nests of cells with nuclear features of papillary thyroid carcinoma.

TALL CELL VARIANT:

Features: [57,58]

- Papillary architecture lined by cells which are three times taller than wider and contain abundant eosinophilic cytoplasm - atleast 50% of the tumor cells should present with these features.
- Older age persons (50-57 years) are commonly affected.
- Extrathyroidal extension is more common.
- Extensive lymphocytic infiltration of the stroma.[59]
- More aggressive form.
- BRAF mutation noted in about 80% of patients.
- CD15 and CEA are expressed compared to papillary thyroid carcinoma.

PTC WITH NODULAR FASCIITIS LIKE STROMA:

This variant is composed of spindle cells arranged in fascicles in a background of vascularised fibrovascular stroma with histiocytes and inflammatory cells. Individual spindle cells have plump nuclei with a finely granular and bland chromatin pattern.

The stromal reaction which is prominent in this variant of papillary thyroid carcinoma gives a fibroadenoma like appearance to the tumor.[60] Histologically, it resembles nodular fasciitis and fibromatosis.[61]

IMMUNOHISTOCHEMISTRY:

Immunohistochemistry is found to be useful in differentiating follicular variant of papillary carcinoma from follicular adenoma and follicular carcinoma. Most useful positive markers are thyroglobulin (except in columnar cell variant), cytokeratin 19, galectin-3 and HBME-1. Other markers that show positivity include cytokeratin 7, high molecular weight keratin demonstrated with 34ßE12, TTF-2, PAX8, S-100 protein, vimentin, EMA, CD15, CD57, alpha1-antichymotrypsin, HER2/neu, insulin-like growth factor 1 and cMet/hepatocyte growth factor receptor. TTF-1, cytokeratin 20 and CEA are negative in these tumors.

MOLECULAR GENETICS:

Activation of the mitogen-activated protein kinase (MAPK) pathway as a result of three different molecular events plays a major role in the molecular genetic feature of papillary thyroid carcinoma.[62]

Molecular events are as follows:[63]

1. RET/PTC rearrangements or the less common TRK rearrangements,

2. BRAF mutations and

3. RAS activating mutations.

These molecular alterations are mutually exclusive. MAPK pathway regulates important cellular functions such as proliferation, differentiation, and survival.

20-40% of papillary carcinomas are due to gene rearrangements, mainly involving RET oncogene, a transmembrane tyrosine kinase receptor which is located in chromosome 10q11.2. RET rearrangements occur in the intron 11 of RET by intrachromosomal inversions of the long arm of chromosome 10 or by inter-chromosomal translocations.[64] This oncogene is unique to thyroid tumors, as this results in medullary carcinoma by activating RET point mutations and also causes papillary carcinoma by somatic RET rearrangements.

RET/PTC are chimeric genes for papillary thyroid carcinoma, which are produced by the in-frame fusion of the entire RET tyrosine-kinase domain with other genes. RET/PTC1 (RET fusion with CCDC6, a.k.a. H4 or D10S170) and RET/PTC3 (RET fusion with NcoA4, a.k.a. RFG, ELE1 or ARA70) – these two genes are the result of intrachromosomal rearrangements constituting about >90%, with RET/PTC1 positive in two-third cases and RET/PTC3 being positive in one-third (but more aggressive in nature), of RET/PTC-positive cases. RET/PTC2 (RET fusion with PRKAR1A) is a gene that is inactivated in patients with Carney complex.

RET/PTC is common in papillary carcinomas of children and young adults. They show positivity in classical papillary carcinoma or microcarcinoma even at low stage of presentation with little proliferative activity. Even low level of RET/PTC rearrangement detection is possible by highly sensitive techniques in papillary carcinomas. The rearrangements can be identified by reverse transcriptase-polymerase chain reaction (RT-PCR) or fluorescent in situ hybridization (FISH).

BRAF mutation:

BRAF activating mutations are the most common molecular genetic alteration occuring in about 30-70% of papillary carcinomas. Oncogenic BRAF activation is also seen to occur in many human cancers like melanoma and colorectal adenocarcinoma.[65] It is a serine/threonine kinase, a member of the

RAF family of protein kinases, which plays a role in the MAPK pathway. The most common mutation is a missense point mutation, caused by transversion of thymidine to adenine in the nucleotide 1799 (T1799A) of exon 15, that results in substitution of valine by glutamic acid at residue 600 (V600E mutation).[65] Other rare BRAF mutants include K601E mutation, paracentric inversion of chromosome 7 and small insertions or deletions close to codon 600.

BRAF mutations are highly specific for papillary thyroid carcinoma and also found in papillary carcinomas found within struma ovarii.[66] It is more commonly seen in classical papillary carcinoma, tall cell variant and oncocytic variant. It is very uncommon in the follicular variant and is found to be absent in follicular neoplasms and medullary carcinoma. BRAF mutations are found to be associated with male gender, older age at diagnosis, extrathyroidal invasion, metastases to lymph nodes and to distant sites, high tumor stage at presentation, tumor recurrence (even for stage I and II disease) and reduced survival.[67]

RAS mutation:

RAS mutations are identified as a marker for follicular-patterned thyroid tumors like follicular adenoma, follicular carcinoma and follicular variant of papillary thyroid carcinoma.[67]

NTRK1 gene:

NTRK1 gene rearrangement is noted in papillary carcinoma of thyroid, constituting about 5%. This gene encodes a transmembrane tyrosine kinase receptor which binds with the nerve growth factor. It is activated by intra- or inter-chromosomal recombination of the NTRK1 locus at chromosome 1q22, fusion of NTRK1 to heterologous genes producing chimeric oncogenes, aberrant expression and also by ligand independent activation of the NTRK1 tyrosine kinase.

PROGNOSIS: [68]

Better prognostic factors:

1. Age less than 40 years - includes children and adolescents.[69]
2. Females.
3. Encapsulated variant.[70]

Poor prognostic factors:

1. Age more than 40 years.
2. Males.
3. Tall cell, follicular and oncocytic variants.
4. Extrathyroidal extension.
5. Larger tumor size.
6. Multicentric tumor.

7. Metastases to distant sites.
8. Poorly differentiated and anaplastic carcinoma.
9. More aggressive nature in patients with aneuploidy and BRAF mutations.

3.9.2) FOLLICULAR CARCINOMA:

Follicular carcinoma is a rare neoplasm constituting about 5-10% of all primary thyroid tumors.[71] Prevalence is more common among elderly females. It spreads via hematogenous route and distant metastasis is common, especially to lungs and bone.

Predisposing factors:

- Higher incidence noted in people living in areas with endemic goitre.
- Iodine deficiency.
- Dyshormonogenesis.
- Irradiation.
- Rarely in a pre-existing follicular adenoma.

Gross:

It usually presents as an encapsulated solitary tumor ranging in size from less than 1 cm to 10 cm. Solid, fleshy and tan to light brown in colour with secondary changes like haemorrhage and cystic degeneration. Lack of discrete capsule and presence of tumor thrombi distending the blood vessels are seen in widely invasive follicular carcinoma. Minimally invasive follicular carcinoma being encapsulated like follicular adenoma is identified by the thicker capsule.

Microscopy:

It shows evidence of follicular cell differentiation but lacks the diagnostic features of papillary carcinoma. Variable histological features and comprises of well-formed closely packed follicles to trabeculae and solid pattern. Poorly formed follicles and cribriform pattern may also be seen. Individual tumor cells are cuboidal or low columnar with hyperchromatic or pale-staining round pleomorphic nuclei with inconspicuous nucleoli. However, the diagnosis and the differentiating feature of follicular carcinoma from follicular adenoma depends on the presence of capsular and vascular invasion.

CRITERIA FOR CAPSULAR INVASION:

1. Complete transgression of the fibrous capsule.
2. Mushroom-shaped bud that has extended beyond the outer contour of the fibrous capsule.
3. Satellite nodule with cytoarchitectural and cellular features identical to that of the main tumor.
4. Tumor bud that has invaded beyond the outer contour of the fibrous capsule.

CRITERIA FOR VASCULAR INVASION:

1. Intracapsular or extracapsular blood vessel with a tumor plug should be lined by endothelium.

2. Tumor bud pushing through the fibrous capsule and protruding into the lumen of a capsular blood vessel.
3. Non-endothelialized vascular tumor plug, if it is accompanied by fibrin thrombus is a criterion for vascular invasion.
4. Involved blood vessel should not be within the tumor.
5. Tumor fragments seen floating in the vascular lumen should not be considered.

Histological subtypes:

Based on the degree of invasiveness, subdivided into two types:

1. Minimally invasive follicular carcinoma.
2. Widely invasive follicular carcinoma.

MINIMALLY INVASIVE FOLLICULAR CARCINOMA:

It is a grossly encapsulated tumor, resembling an adenoma of embryonal or fetal type. Cut surface is solid and fleshy. Vascular and capsular invasion should be present. Endothelial markers useful in identification are CD31 (more preferred), factor VIII-related antigen, Ulex europaeus and Fli-1.

WIDELY INVASIVE FOLLICULAR CARCINOMA:

Mostly encapsulation will not be present. If it presents as a grossly encapsulated tumor, widespread invasion of four or more blood vessels and/or infiltration into adjacent thyroid tissue should be present.

MOLECULAR GENETICS:[72]

Follicular carcinoma is found in association with RAS, PTEN and PIK3CA mutations. Most common is the RAS mutation at codon 61 of NRAS.

Activation of the PI3K/PTEN/AKT pathway due to loss of function mutations in PTEN gene is seen in patients with Cowden syndrome,[73] Carney complex type I and Werner syndrome (adult progeria). In these conditions, follicular carcinoma is seen as a disease manifestation.

20% per chromosome arm show Loss Of Heterozygosity (LOH) in follicular carcinoma when compared to 5% in follicular adenoma and 2.5% in papillary carcinoma.[74]

PAX-8 encodes a transcription factor which plays a vital role in thyroid development and differentiation and PPARγ encodes peroxisome proliferator–activated receptor. The PAX8/PPARγ rearrangement,[75] caused by the t(2;3)(q13;p25) fuses PPARγ at 3p25 with PAX8. This rearrangement is not specific for follicular carcinoma but is also seen in follicular adenomas and follicular variant of papillary carcinoma.[76] In follicular carcinomas, PAX8/ PPARγ rearrangement is common in females, young age, tumors with high cellularity and invasive features. FISH, RT-PCR, and immunohistochemistry with anti-PPARγ antibodies are useful in identifying this rearrangement.

IMMUNOHISTOCHEMISTRY:

Matrix metalloproteinases, MCM2 (a cell proliferation marker) and human telomerase reverse transcriptase (hTERT) are most useful markers whose expression is helpful in differentiating follicular carcinomas from follicular adenomas or hyperplastic nodules.[77,78] Other reactive markers are Thyroglobulin, TTF-1, low molecular weight keratin, galectin-3, EMA, and basement membrane components like laminin and type IV collagen.[79]

3.9.3) POORLY DIFFERENTIATED CARCINOMA:

Tumors presenting with histological features intermediate between well-differentiated thyroid carcinomas and undifferentiated carcinomas are termed as poorly differentiated carcinomas.[80] Insular carcinoma,[81] primordial cell carcinoma,[82] and poorly differentiated papillary thyroid carcinoma are other names used for this carcinoma. They usually arise denovo but can also arise by transformation of differentiated carcinoma or they can even transform into undifferentiated carcinoma. Females and elderly people about 60 years of age are more commonly affected. Extrathyroidal extension, recurrence, metastases to lymph node and to distant sites by hematogenous route are common. They concentrate radioiodine – this property is useful in diagnostic and therapeutic purposes.[83]

Gross:

It is a solid, firm tumor with a grey white cut surface, usually infiltrative but may also be partially encapsulated. Haemorrhage and necrosis are common.

Microscopy:

Tumor cells are arranged in a nesting ('insular') pattern with retraction artifact, diffuse solid sheets, trabecular or microfollicular pattern. Individual cells are small, uniform with round hyperchromatic or vesicular nuclei with indistinct nucleoli and scant cytoplasm. Mitotic activity is variable. Coagulative necrosis is common resulting in a peritheliomatous appearance.

IMMUNOHISTOCHEMISTRY:

Tumor cells are positive for thyroglobulin, TTF-1, PAX-8 and cyclin-D1. Shows negative staining for calcitonin, a feature to distinguish from medullary carcinoma due to presence of insular pattern. Has decreased expression of the cyclin-dependent kinase inhibitor p27 and increased Ki-67 index.[84]

MOLECULAR GENETICS:

TP53 and ß-catenin mutations are common.[85,86]

PROGNOSIS:

Advanced age (≥45 years), larger tumor size (≥4 cm), extrathyroidal extension, presence of metastasis, presence of an undifferentiated carcinoma component, and immunohistochemical expression of insulin-like growth factor–II messenger RNA binding protein-3 (IMP-3) are considered as poor prognostic factors.

3.9.4) UNDIFFERENTIATED / ANAPLASTIC CARCINOMA:

Anaplastic carcinoma constitutes about 2-5% of all thyroid cancers.[87]

Elderly females more than 70 years of age are more commonly affected. They are rapidly enlarging aggressive tumor mass seen clinically associated with hoarseness, dysphagia, and dyspnoea. Regional lymph node and distant metastases are common at presentation itself. Hence, most of the patients die within a year, usually in less than 6 months due to involvement of vital structures in the neck.

Gross:

Almost the entire normal thyroid parenchyma is replaced by fleshy, tan to white, solid tumor mass with extensive areas of haemorrhage and necrosis. It commonly invades adjacent soft tissue.

Microscopy:

Presents with variable histological features. Tumor cells are of sarcomatoid type with spindle cells and pleomorphic giant cells or occasionally squamoid type. Epithelial looking tumor cells are arranged in clusters and sheets. Individual tumor cells are large polygonal or round cells with pleomorphic nuclei and moderate amount of eosinophilic cytoplasm. Sarcomatoid component shows fascicular or storiform pattern with abundant infiltration by neutrophils, prominent vascularization and differentiation into bone, cartilage and skeletal muscle.[88] Osteoclast-like multinucleated giant cells are non-neoplastic and represent reactive cells of monocytic/histiocytic lineage derived from mononuclear cells. Extensive haemorrhage and necrosis are seen.

MORPHOLOGIC VARIANTS:

1. Angiomatoid Variant.
2. Osteoclastic Variant.
3. Rhabdoid Variant.
4. Lymphoepithelioma-like Carcinoma.
5. Pauci-cellular Variant.
6. Carcinosarcoma.
7. Adenosquamous Carcinoma.
8. Squamous Cell Carcinoma.

IMMUNOHISTOCHEMISTRY:

Keratin is positive in epithelial component in 50-100%[89] and PAX-8 in 76% of cases.[90] Vimentin is positive in the spindle cell component. Focal EMA and CEA positivity may be seen in the squamoid type. Thyroglobulin and TTF-1 are negative.

MOLECULAR GENETICS:

Most common is the TP53 mutation with diffuse nuclear accumulation throughout the tumor.[91] Other mutations include ß-catenin mutation, RAS and BRAF mutations.

PROGNOSIS:

Overall it has a very poor prognosis. Older age (≥70 years), leukocytosis, larger tumor size (>5 cm), extrathyroidal extension and distant metastasis are considered poor prognostic factors.[92]

3.9.5) MEDULLARY CARCINOMA:

It is a malignant tumor, derived from parafollicular C-cells of thyroid.[93] It secretes calcitonin. Sporadic and inherited forms are present. Majority are of sporadic origin constituting about 80%, shows unilaterality and cold on thyroid scan. Adults with mean age around 45 years are affected. Hereditary forms may occur as a part of multiple endocrine neoplasia type 2a (MEN 2a), type 2b (MEN 2b) or isolated as familial medullary thyroid carcinoma syndrome,

(FMTC).[94,95] Medullary carcinoma is the first manifestation of the syndrome. It usually occurs in younger patients around 35 years of age, multicentric, bilateral and presence of C-cell hyperplasia. RET gene located on chromosome 10q11.2 is affected and causes inherited form.[96]

Gross:

It is non-encapsulated but well circumscribed solid, firm tumor. Cut surface is grey-white to tan, yellowish or reddish brown in colour. This lesion is usually situated in the middle third of the lateral lobe, where the density of C-cells will be highest. Larger tumors may have haemorrhage and central necrosis. Medullary microcarcinoma refers to medullary carcinoma with greatest diameter of the tumor less than or equal to 1cm.

Microscopy:

Histological patterns are variable. Tumor cells are arranged in sheets, nests or islands separated by prominent delicate fibrovascular septa, hyalinised collagen and amyloid. Other patterns like whorling, trabecular, pseudopapillary, rosette, tubular, microglandular or cribriform pattern may also be present. Individual cells are round, polygonal or plump spindle cells with finely stippled nuclear chromatin and inconspicuous nucleoli. 80-85% of cases have amyloid which are pink amorphous material. Increase in the vascular stroma is the key feature.

Histological variants:

1. Glandular or Follicular.
2. Oxyphilic or oncocytic.
3. Giant Cell or Anaplastic.
4. Clear Cell.
5. Spindle Cell.
6. Melanotic or pigmented.
7. Squamous.
8. Papillary or pseudopapillary.
9. Small Cell.
10. Pseudoangiosarcomatous.
11. Neuroblastoma-like.
12. Hyalinizing Trabecular Adenoma-like.
13. Carcinoid-like.
14. Paraganglioma-like.

IMMUNOHISTOCHEMISTRY:

Tumor shows positivity for calcitonin (almost all tumor cells),[97] cytokeratin, pan-neuroendocrine marker, CEA and TTF-1. Calcitonin-poor medullary carcinoma is highly aggressive than calcitonin-rich tumor. Shows negativity for thyroglobulin.[98] Immunohistochemistry is the confirmatory method to make a diagnosis of medullary carcinoma.

PROGNOSIS:

Age and stage are the most important factors.

Better prognostic factors:

- Females.
- MEN-2A type of medullary carcinoma.
- Medullary microcarcinoma.

Poor prognostic factors:

- Age more than 45 years.
- Presence of lymph node and distant metastasis.
- MEN-2B type of medullary carcinoma.
- Histologic features: High mitotic count (more than 1 per 25 high-power fields), small cell variant, necrosis, squamous metaplasia and absence of amyloid.[99]
- Calcitonin poor medullary carcinoma.
- Presence of a somatic RET gene mutation.

3.10) IMMUNOHISTOCHEMISTRY IN THYROID NEOPLASMS:

Histopathology remains the mainstay of diagnostic modality for thyroid lesions. However, with the advent of ancillary techniques, immunohistochemistry is now being used as an aid in diagnosis of thyroid neoplasms, to overcome difficulty arising due to overlap of morphological features.

The follicular patterned thyroid lesions like follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma needs to be differentiated as the treatment modality, outcome and prognosis varies. Characteristic nuclear features of papillary carcinoma like nuclear overlapping, nuclear grooves and intranuclear pseudo-inclusions may also be present in some cases of multinodular goitre with papillary hyperplasia and hyalinising trabecular adenoma.

In order to overcome these diagnostic problems, immunohistochemical markers are used. Markers used are thyroglobulin, GALECTIN-3, CD 56, TTF-1, cytokeratin-19, HBME-1 and PAX-8.

GALECTIN-3:[100–102]

Galectin-3 belongs to a group of lectin family. It is a protein that is encoded by a single gene LGALS3, located on chromosome 14, locus q21-q22 in humans. It is a 31-kDa galactosidase binding lectin that has affinity for beta-

galactoside containing intracellular, extracellular and cell surface associated glyco-conjugates.

It is predominantly expressed in the cytoplasm of epithelial cells and immune cells but also found to be expressed in thyroid, breast, colon, macrophages and activated endothelial cells. It regulates cell-cell and cell-matrix interaction, cell growth, angiogenesis, apoptosis, macrophage activation, inflammatory response and malignant transformation.

Immunohistochemical staining pattern of galectin-3:

Specific staining of more than 5% of the tumor cells with whatever may be the intensity of staining like slight, moderate or intense, the result is interpreted as positive.[101,103] The intensity of staining was graded into four scales from 0 to 3 and the proportion of stained cells scored as 1+ to 3+.

Intensity grading of stained cells:

0 = no staining.

1+ = slight staining.

2+ = Moderate staining.

3+ = Intense staining.

Proportion scoring of stained cells (percentage of tumor cells expressing galectin-3):

1+ = less than 5 % of cells.

2+ = 5 to 50 % of cells.

3+ = more than 50 % of cells.

Cytoplasmic galectin-3 has been found to be expressed only in carcinomas and hence, considered as an evidence of malignancy. It is now increasingly being used as a diagnostic marker, distinguishing benign from malignant thyroid lesions.

CD56:

CD56 antigen (NCAM – Neural Cell Adhesion Molecule, Leu19) belonging to a group of immunoglobulin superfamily, is a membrane binding glycoprotein which plays a role in cell-cell adhesion. It is found to be expressed in a wide variety of cell types, predominantly cells of neural and mesenchymal origin and also in endocrine cells.[104,105] It is also normally expressed in Natural Killer (NK) cells, activated T cells, large granular lymphocytes and follicular epithelial cells of thyroid gland.[106]

Cases are considered to be CD56 positive, if more than or equal to 10% of the tumor cells show membranous positivity. Focal membranous or cytoplasmic positivity in less than 10% of tumor cells are considered negative.

Loss of CD56 expression is considered a specific and sensitive marker of papillary carcinoma of thyroid.[107,108]

THYROGLOBULIN:

Thyroglobulin is a 660kDa, glycosylated dimeric protein produced by the follicular epithelial cells of the thyroid. It is a precursor for the synthesis of thyroid hormones. It is a marker for thyroid follicular epithelial cell differentiation and hence, used as a tumor marker in papillary carcinoma, follicular adenoma, follicular carcinoma and poorly differentiated carcinoma. It is negative in medullary and anaplastic thyroid carcinoma.

TTF-1:

It is also known as NK2 homeobox 1 (NKX2-1) or thyroid specific enhancer binding protein. It is a 38kDa nuclear protein encoded by NKX2-1 gene in humans. It regulates transcription of thyroid specific genes and also genes for lung and diencephalon. It is usually expressed in thyroid follicular cells, parafollicular C-cells and type II pneumocytes in lung. It is usually positive in adenocarcinomas and small cell carcinomas of lung. It is also positive in thyroid cancers and is used for monitoring of metastasis and recurrence.[109]

HBME-1:

Marker of mesothelial cells, named after the laboratory of Dr. **H**ector **B**attifora and **ME**sothelioma. It is a monoclonal antibody that acts mainly against the antigen on the mesothelial cell membrane. It is positive in papillary and follicular carcinoma of thyroid.[110]

CYTOKERATIN-19:

Cytokeratin-19 is a 40kDa protein that is encoded by the KRT19 gene. It belongs to a group of keratin family and Keratin 19 is a type I keratin. It is an intermediate filament protein that is responsible for the structural integrity of epithelial cells. They are clustered in a region of chromosome 17q12-q21. It is used as a diagnostic and sensitive marker of papillary thyroid carcinoma.[111] It is also expressed in basal keratinocytes, sweat gland, gastrointestinal tract, mammary gland ductal and secretory cells, epithelium of ectocervix and urothelium.

PAX8:

Paired box gene 8, also known as **PAX8**, is a protein which is encoded by the PAX8 gene in humans. This gene is a member of the paired box family of transcription factors. This nuclear protein is involved in thyroid follicular cell development and expression of thyroid-specific genes. PAX8 releases the hormones important for growth regulation, brain development and metabolism.

It also functions in very early stages of renal organogenesis, the mullerian system and the thymus. Additionally, PAX8 is expressed in the renal excretory system, epithelial cells of the endocervix, endometrium, ovary, fallopian tube, seminal vesicle, epididymis, pancreatic islet cells and lymphoid cells. It shows positive reactivity in papillary carcinoma, follicular neoplasms, poorly differentiated carcinoma, medullary carcinoma and undifferentiated carcinoma. It is also expressed in B cell lymphomas and renal cell carcinoma.

4. MATERIALS AND METHODS

Study population:

Cases that were diagnosed as thyroid neoplasms using Haematoxylin and Eosin stain.

Study design:

Prospective study.

Study period:

January 2017 to June 2018.

Study place:

Coimbatore Medical College Hospital, Coimbatore.

Sample size:

A total number of 30 cases of surgically resected thyroid neoplasms. Out of 30 cases, 8 cases were benign tumors, which included 7 cases of follicular adenoma (n=7) and 1 case of Hurthle cell adenoma (n=1) and 22 cases were malignant thyroid tumors, which included 18 cases of papillary thyroid carcinoma (n=18) - of which 4 were follicular variant of papillary carcinoma, 3 cases of follicular carcinoma (n=3) and 1 case of minimally invasive follicular carcinoma (n=1).

Inclusion criteria:

a) All cases of operated thyroid tumors received in the department of pathology.
b) Patients in all age groups.
c) Both sexes are included.

Exclusion criteria:

a) Ill fixed or autolysed specimens at the time of receiving.
b) Recurrent cases after treatment.

Materials required:

a) Donor blocks containing formalin fixed paraffin embedded tissue of thyroid tumors.
b) Haematoxylin and Eosin stained sections of tissues.
c) Poly 1-lysine coated slides for holding sections for IHC.
d) Chemicals used for preparing antigen retrieval solution and wash buffer solution.
e) Microwave oven for antigen retrieval.
f) Primary antibodies used in this study – Galectin-3 and CD56.
g) Secondary universal kit for immunohistochemistry.
h) Microscope to view the slides.

METHODOLOGY:

A brief clinical data of the patient such as age, sex, clinical diagnosis and surgical procedure were collected from the clinical case records. All those 30 specimens selected were fixed in 10% neutral buffered formalin, embedded in paraffin by clearing and dehydrating the tissue sections with increasing concentrations of alcohol and diluting in an organic solvent, in order to harden the tissue to be cut into thin sections and then a section of 4 microns thickness was made with a microtome. Slides were then stained with routine Haematoxylin and Eosin stains.

HEMATOXYLIN AND EOSIN STAINING:

REAGENTS USED:

1. Haematoxylin solution – ERHLICH's haematoxylin.
2. Eosin Y – 1% solution.
3. Acid alcohol – 1% solution.

PROCEDURE:

1. Sections were deparaffinized by immersing in xylene for 30 seconds.
2. Place the sections in isopropyl alcohol for 15 minutes.
3. Washed in running tap water.
4. Stained using Erhlich's haematoxylin for 10-15 minutes.
5. Differentiated with 1% acid alcohol - 2 to 3 dips.

6. Blueing of the sections for 10 minutes.
7. Counterstained with 1% eosin solution - 3 to 4 dips.
8. Rinsed in running tap water.
9. Air dry the slides.
10. Mounted with DPX mountant (non-aqueous medium).

Haematoxylin stains the nuclei of the cell blue and Eosin stains the cytoplasm pink. All slides were then reviewed.

IMMUNOHISTOCHEMISTRY:

PRINCIPLE:

It is a two-step indirect method. First step is the binding of primary antibody to specific epitopes. Secondly, a colorimetric reaction occurs to detect the binding. By this technique, antigens in cells and tissues are detected.

Sections of 5 microns thickness are mounted on poly 1-lysine coated glass slides and incubated at 60-70 degree Celsius for 1 hour. The paraffin embedded sections are dewaxed. Then antigen retrieval done by heating the sections in microwave oven in an aqueous solution. This step will recover full antigenicity. Sections are then treated with peroxidase block (blocks endogenous peroxidase) and power block (blocks non-specific protein-protein interactions) subsequently.

REAGENTS USED:

1. Antigen retrieval solution.
2. Peroxidase block - 3% hydrogen peroxide in water.
3. Primary antibodies - GALECTIN-3 and CD56.
4. Poly-HRP reagent.
5. Chromogen - DAB (3,3-DiAminoBenzidine).
6. DAB buffer substrate.
7. Counterstain - Ehrlich's haematoxylin.

BUFFER PREPARATION:

TRIS EDTA BUFFER: pH = 9

Tris buffer salt	- 6.05 gm.
Disodium EDTA	- 0.744 gm.
Distilled water	- 1 litre.

TRIS WASH BUFFER: pH = 7.6

Tris buffer salt	- 0.605 gm.
Sodium chloride	- 8 gm.
1N Hydrochloric acid	- 4 ml.
Distilled water	- 1 litre.

PROCEDURE:

1. Incubated sections were deparaffinized in 3 changes of xylene for 10 minutes each.
2. Dehydrated in absolute alcohol by 3 changes for 5 minutes each.
3. Sections are then washed in tap water for 5 minutes.
4. Rinsed in distilled water for 2 minutes.
5. Antigen retrieval was done by placing the slides in Tris-EDTA buffer solution in microwave oven, for 10 minutes at medium temperature followed by 10 minutes at high temperature.
6. Cooled to room temperature and then rinsed in distilled water for 5 minutes.
7. Washed in Tris wash buffer – 2 changes for 5 minutes each.
8. Sections are treated with peroxide block for 10 minutes.
9. Washed in Tris wash buffer – 2 changes for 5 minutes each.
10. Primary antibodies – GALECTIN-3 and CD56 (from the manufacturer PathnSitu) were applied and left for 2 hours.
11. Washed in Tris wash buffer – 3 changes for 5 minutes each.
12. Application of HRP - Polymerase for 30 minutes.
13. Washed in Tris wash buffer – 3 changes for 5 minutes each.
14. Application of DAB chromogen (1drop) and DAB buffer substrate (1ml) for 5 to 8 minutes.

15. Rinsed in distilled water.
16. Counterstained with Ehrlich's haematoxylin – 1 dip for 30 seconds to stain the background.
17. Washed in running tap water for 5 minutes.
18. Air dry the slides.
19. Mounted with DPX mountant.
20. Observation and grading done under light microscope.

PRECAUTIONS TO BE FOLLOWED:

- All glasswares used should be clean and dry.
- Reagents like peroxidase block, primary antibody, HRP polymerase and DAB chromogen should be stored at an ideal temperature of 4-6 degree Celsius.
- Freshly prepared buffer solutions should be used and their pH maintained.
- Slides should NEVER be allowed to dry during the procedure.
- DAB chromogen should be carefully handled as it is carcinogenic.

PRIMARY ANTIBODIES USED:

Antibodies	Clone	Dilution	Manufacturer	Antigen retrieval buffer	Buffer pH
Galectin-3	9C4	Prediluted	PathnSitu	Tris EDTA	9
CD56	123C3	Prediluted	PathnSitu	Tris EDTA	9

SOURCE: Mouse Monoclonal antibody.

INTERPRETATION OF IMMUNOHISTOCHEMISTRY:

GALECTIN-3:

Cytoplasmic Galectin-3 if expressed in more than 5% of tumor cells is considered as **POSITIVE**, regardless of the staining intensity.

Intensity grading of stained cells:

0 = no staining.

1 = slight staining.

2 = Moderate staining.

3 = Intense staining.

Proportion scoring of stained cells (percentage of tumor cells expressing galectin-3):

1+ = less than 5 % of cells.

2+ = 5 to 50 % of cells.

3+ = more than 50 % of cells.

CD56:

Intensity grading of stained cells:

0 = no staining.

1 = slight staining.

2 = Moderate staining.

3 = Intense staining.

Proportion scoring of stained cells (Percentage of tumor cells expressing CD56):

0 = less than 10 % of cells.

1+ = 10 to 25 % of cells.

2+ = 25 to 50 % of cells.

3+ = more than 50% of tumor cells.

CD56 marker cell membrane staining, if expressed in more than or equal to 10% of tumor cells is considered as **POSITIVE**.

5. OBSERVATIONS AND RESULTS

This is a prospective study comprising of 30 cases of thyroid neoplasms conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore during the period from January 2017 to June 2018. Permission was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore and then the study was conducted.

Histopathological examination was carried out for the 30 cases of thyroid neoplasms and they were evaluated for the immunohistochemical staining pattern and expression of the two markers Galectin-3 and CD56. Results were analysed and compared with the previous literature.

TABLE 1: MEAN AGE IN THE PRESENT STUDY

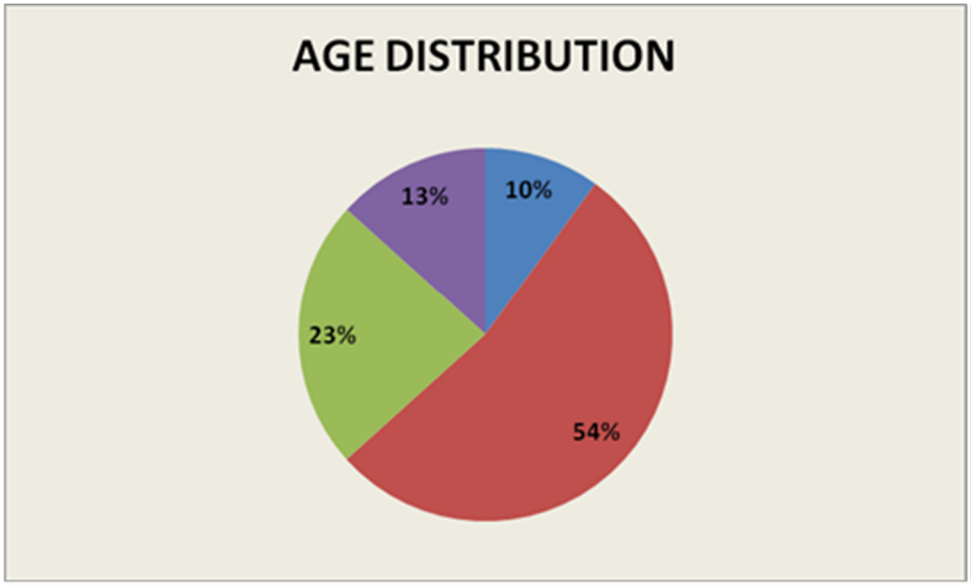
	Number of Cases	**Minimum**	**Maximum**	**Mean ± SD**
AGE (Years)	30	17	67	40.23

In the present study on thyroid neoplasms, the mean age group affected was around 40 years, with minimum age being 17 years and maximum age being 67 years of age.

TABLE 2: AGE DISTRIBUTION IN THE PRESENT STUDY

AGE (in years)	FREQUENCY	PERCENTAGE (%)
<20	3	10.0
21-40	16	53.3
41-60	7	23.3
>61	4	13.3
TOTAL	30	100.0

CHART 1: AGE DISTRIBUTION IN THE PRESENT STUDY

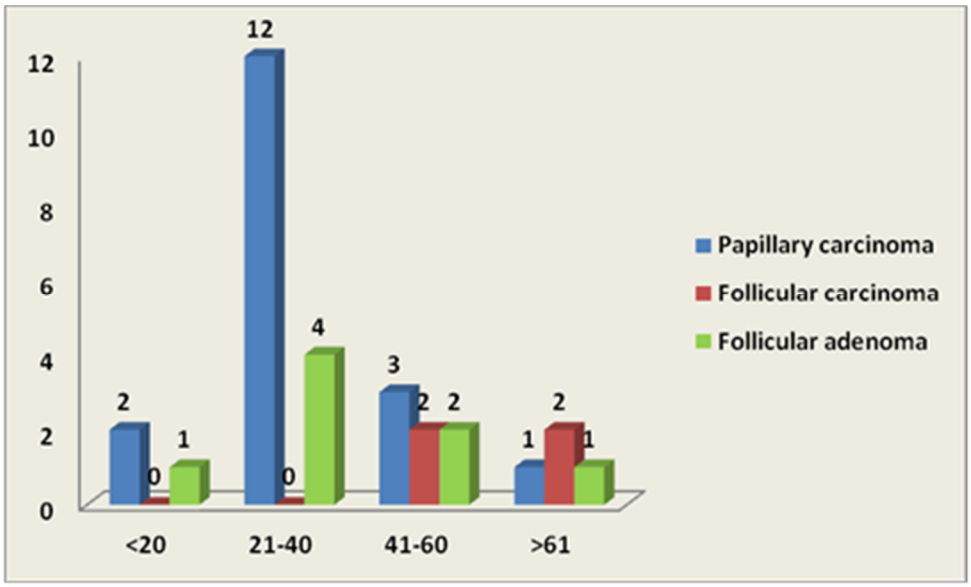


In the present study, 54% of the thyroid neoplasms were found in the age group of 21-40years (16 out of 30 cases), followed by 23% between 41-60 years (7 out of 30 cases), 13% were more than 61 years (4 out of 30 cases) and 10% were less than 20 years of age (3 out of 30 cases).

TABLE 3: AGE DISTRIBUTION IN DIFFERENT TYPES OF THYROID NEOPLASMS

AGE (years)	Papillary carcinoma (n=18)	Follicular carcinoma (n=4)	Follicular adenoma (n=8)
<20	2	0	1
21-40	12	0	4
41-60	3	2	2
>61	1	2	1

CHART 2: AGE DISTRIBUTION IN DIFFERENT TYPES OF THYROID NEOPLASMS



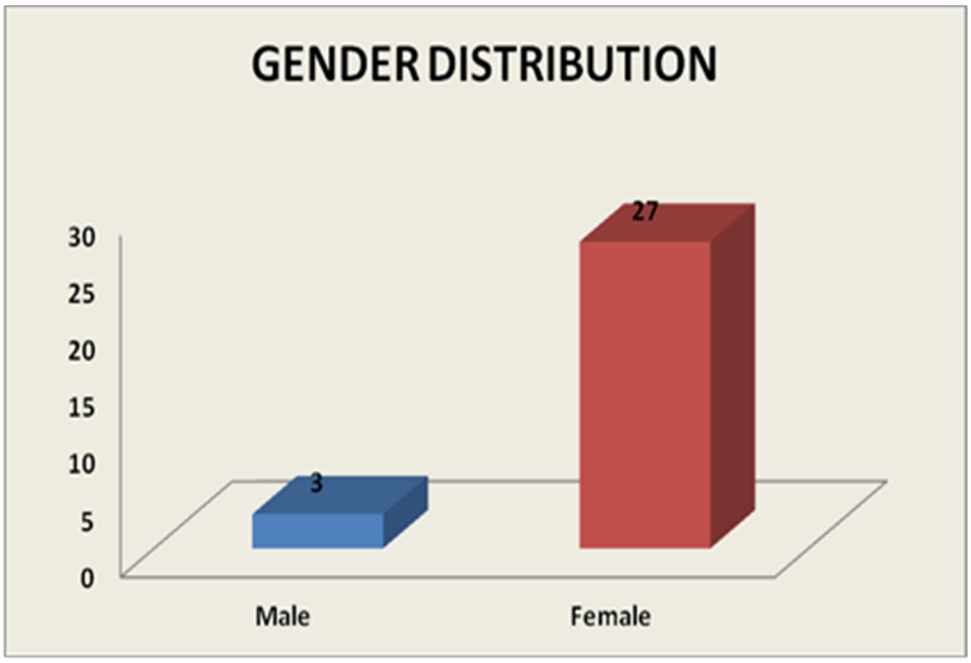
Age (in years)

This diagram depicts the age distribution in our study (n=30) in different types of thyroid neoplasms based on histopathological diagnosis. Younger age group between 21-40 years are more commonly affected, both by malignant (Papillary carcinoma, n=12) and benign (Follicular adenoma, n=4) thyroid neoplasms.

TABLE 4: GENDER DISTRIBUTION IN THE PRESENT STUDY

GENDER	FREQUENCY	PERCENTAGE (%)
Male	3	10.0
Female	27	90.0
TOTAL	30	100.0

CHART 3: GENDER DISTRIBUTION IN THE PRESENT STUDY

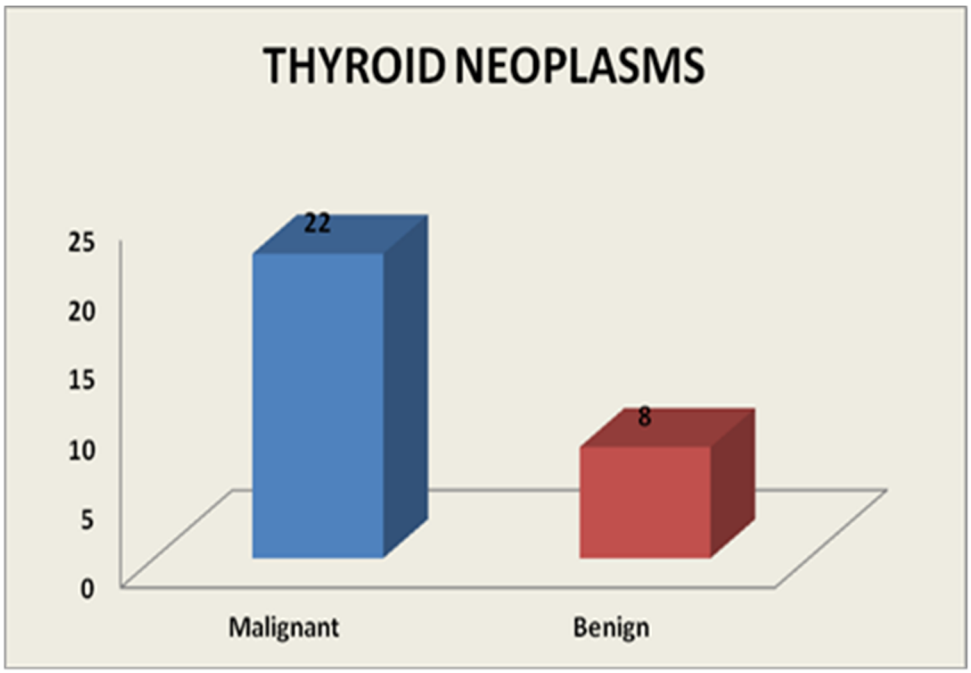


In the present study, females are more commonly affected (27 out of 30 cases) by thyroid neoplasms than males (3 cases), constituting about 90% of the total 30 cases.

TABLE 5: DISTRIBUTION OF NEOPLASMS

TYPES	FREQUENCY	PERCENTAGE (%)
Malignant	22	73.3
Benign	8	26.7
TOTAL	30	100.0

CHART 4: DISTRIBUTION OF NEOPLASMS

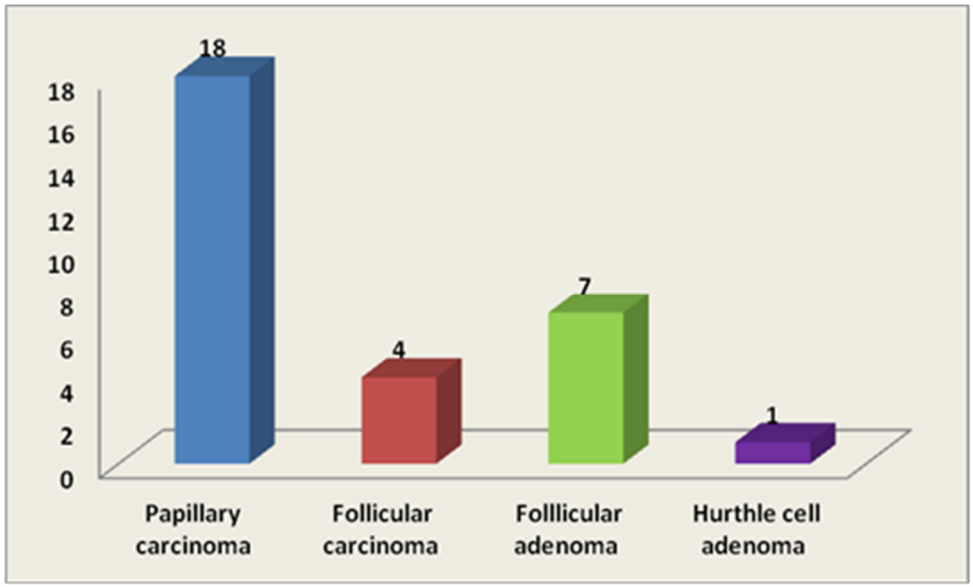


Out of the 30 cases of thyroid neoplasms studied, 22 cases were malignant (73.3%) and 8 cases were benign (26.7%).

TABLE 6: DISTRIBUTION BASED ON HISTOPATHOLOGICAL DIAGNOSIS

HPE DIAGNOSIS	FREQUENCY	PERCENTAGE (%)
Papillary carcinoma	18	60.0
Follicular carcinoma	4	13.4
Follicular adenoma	7	23.3
Hurthle cell adenoma	1	3.3
TOTAL	30	100.0

CHART 5: DISTRIBUTION BASED ON HISTOPATHOLOGICAL DIAGNOSIS

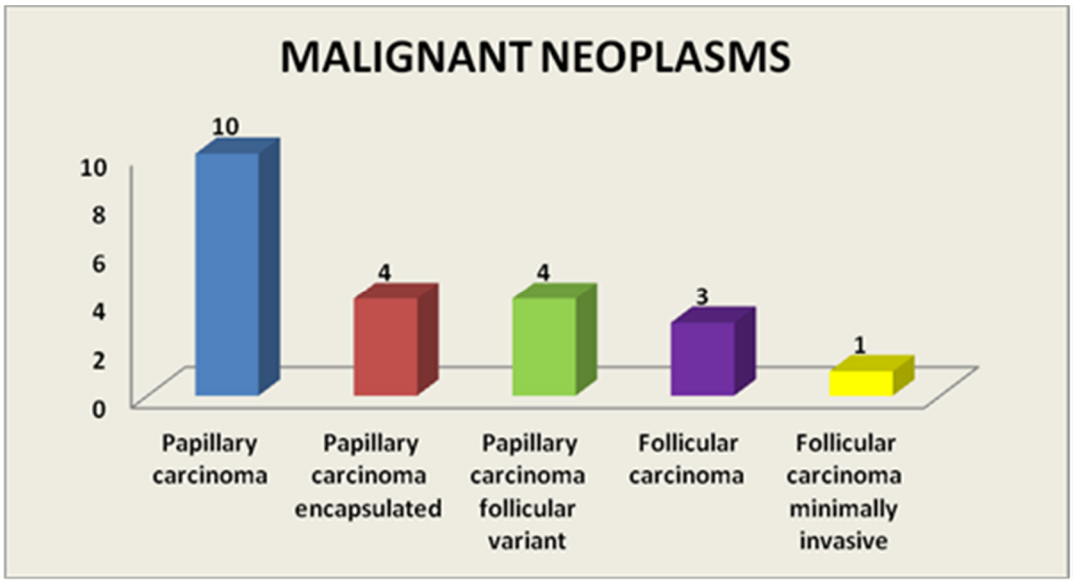


This diagram depicts the distribution of thyroid neoplasms based on histopathological diagnosis. Majority were cases of papillary carcinoma (18 out of 30 cases) accounting to about 60%, followed by follicular adenoma (7 cases – 23.3%).

TABLE 7: DISTRIBUTION OF MALIGNANT THYROID NEOPLASMS

NEOPLASMS	FREQUENCY	PERCENTAGE (%)
Papillary carcinoma	10	45.5
Papillary carcinoma encapsulated	4	18.2
Papillary carcinoma - follicular variant	4	18.2
Follicular carcinoma	3	13.6
Follicular carcinoma minimally invasive	1	4.5
TOTAL	30	100.0

CHART 6: DISTRIBUTION OF MALIGNANT THYROID NEOPLASMS

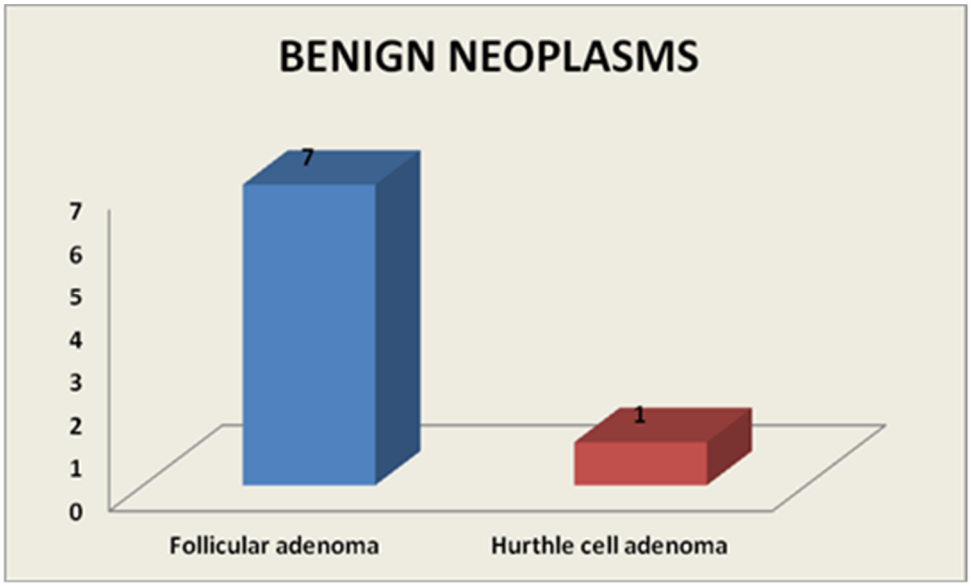


Of the total 18 cases of papillary carcinoma in our study, 10 cases were classical type, 4 cases were encapsulated type and 4 cases were follicular variant of papillary carcinoma. Of the 4 cases of follicular carcinoma – one case was minimally invasive follicular carcinoma.

TABLE 8: DISTRIBUTION OF BENIGN THYROID NEOPLASMS

NEOPLASMS	FREQUENCY	PERCENTAGE (%)
Follicular adenoma	7	87.5
Hurthle cell adenoma	1	12.5
TOTAL	30	100.0

CHART 7: DISTRIBUTION OF BENIGN THYROID NEOPLASMS

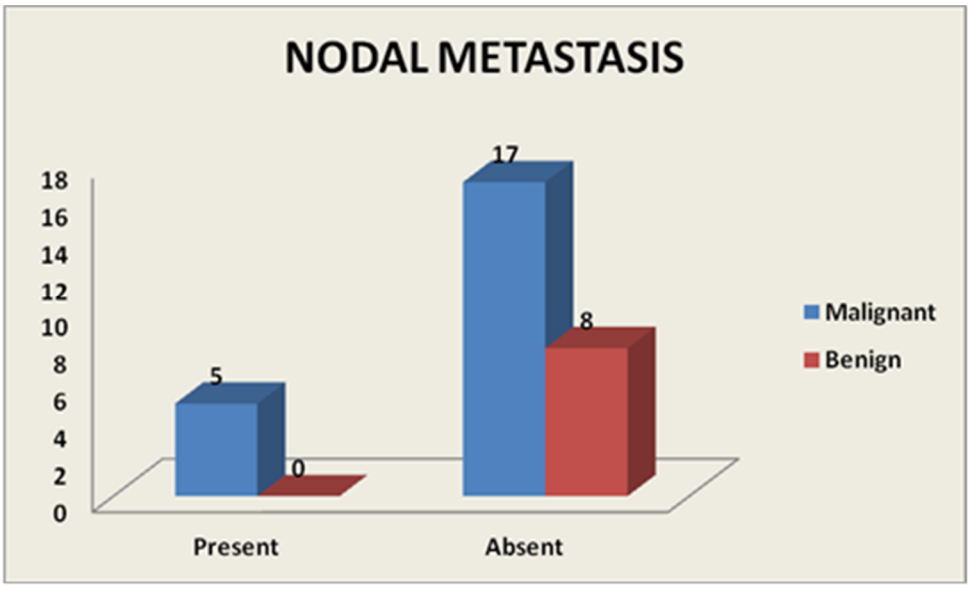


Of the 8 cases of benign thyroid neoplasms in our study, 7 cases (87.5%) were follicular adenoma and 1 case was Hurthle cell adenoma.

TABLE 9: LYMPH NODE METASTASIS IN THYROID NEOPLASMS

NEOPLASMS	METASTASIS	
	Present	**Absent**
Malignant	5(22.7%)	17(77.3%)
Benign	0(0.0%)	8(100.0%)

CHART 8: LYMPH NODE METASTASIS IN THYROID NEOPLASMS



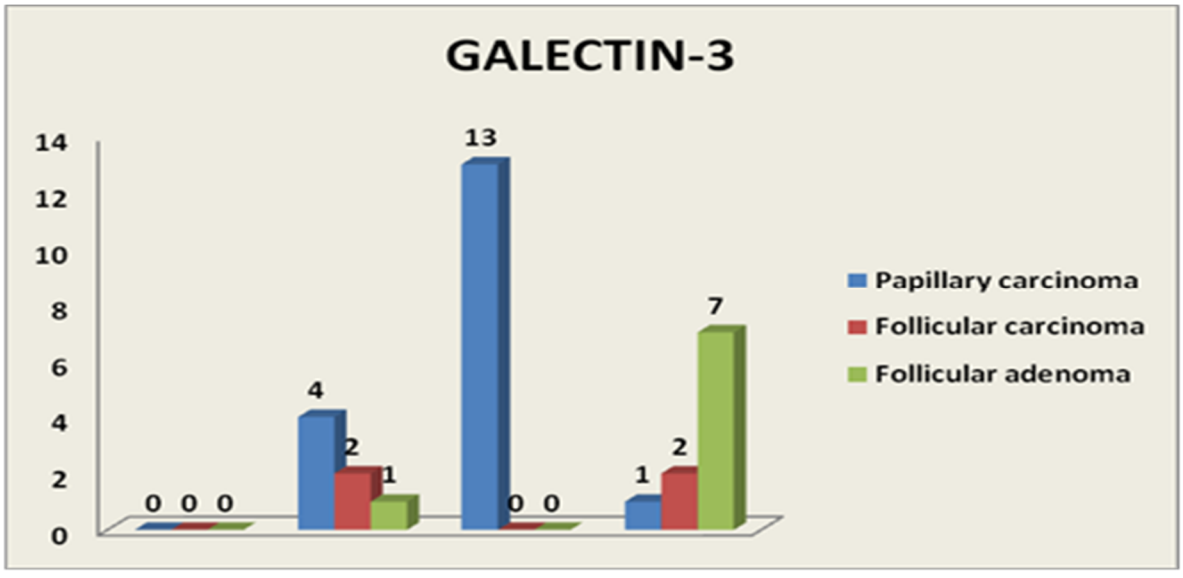
In our study on 30 cases of thyroid neoplasms, 5 cases showed lymph node metastasis in malignancy (5 out of 22 malignant cases = 22.7%) and there was no metastasis noted in the remaining 17 cases of malignant and all the 8 cases of benign neoplasms.

TABLE 10: IMMUNOHISTOCHEMICAL SCORING OF GALECTIN-3 IN THYROID NEOPLASMS

GALECTIN-3	Papillary carcinoma (n=18)	Follicular carcinoma (n=4)	Follicular adenoma (n=8)
1+	0(0%)	0(0%)	0(0%)
2+	4(22.2%)	2(50%)	1(12.5%)
3+	13(72.2%)	0(0%)	0(0%)
Negative	1(5.6%)	2(50%)	7(87.5%)

STATISTICALLY SIGNIFICANT (P<0.05)

CHART 9: IMMUNOHISTOCHEMICAL SCORING OF GALECTIN-3 IN THYROID NEOPLASMS



1+ 2+ 3+ Negative

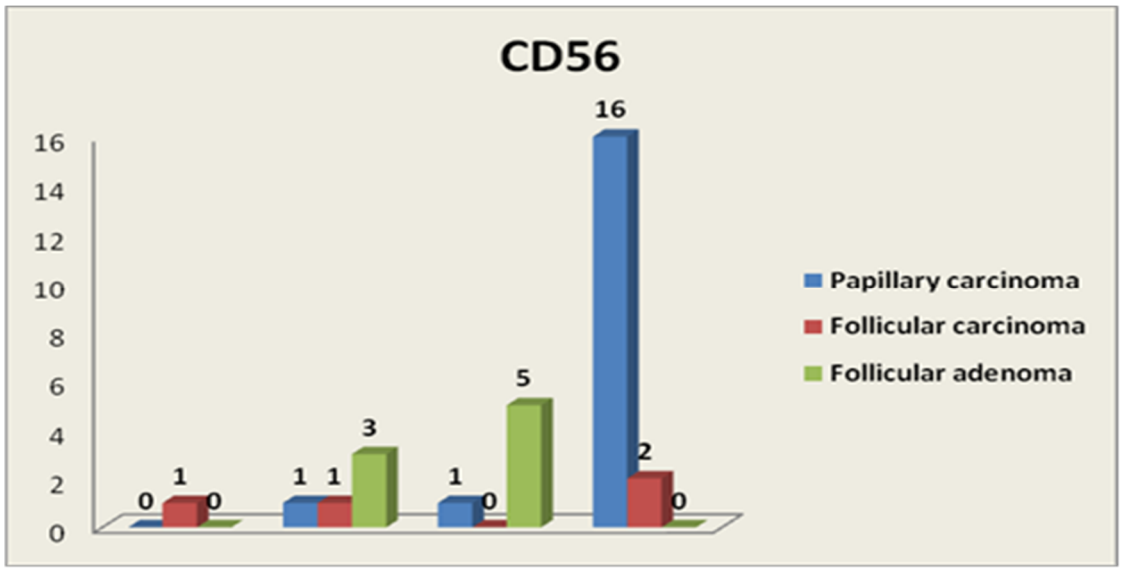
In our study, 13 cases out of the 18 cases of papillary carcinoma showed strong and diffuse 3+ Galectin-3 positivity (72.2%), followed by 2+ positivity in 4 cases (22.2%) and one case showed negative staining with Galectin-3. In follicular carcinoma, 2 cases out of 4 cases (50%) showed 2+ positivity with Galectin-3. In follicular adenoma, 7 out of 8 cases (87.5%) showed negativity with Galectin-3 and 1 case (12.5%) showed 2+ positive expression.

TABLE 11: IMMUNOHISTOCHEMICAL SCORING OF CD56 IN THYROID NEOPLASMS

CD56	Papillary carcinoma (n=18)	Follicular carcinoma (n=4)	Follicular adenoma (n=8)
1+	0(0%)	1(25%)	0(0%)
2+	1(5.6%)	1(25%)	3(37.5%)
3+	1(5.6%)	0(0%)	5(62.5%)
Negative	16(88.8%)	2(50%)	0(0%)

STATISTICALLY SIGNIFICANT (P<0.05)

CHART 10: IMMUNOHISTOCHEMICAL SCORING OF CD56 IN THYROID NEOPLASMS



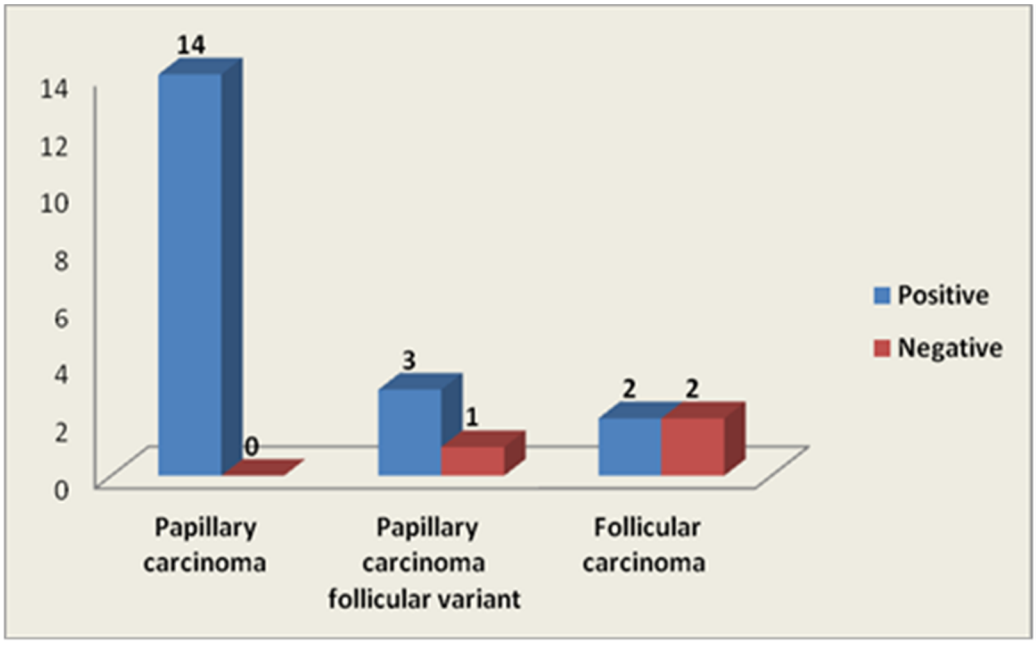
In our study, CD56 expression was negative in 16 out of the 18 cases (88.8%) of papillary carcinoma and 2 out of 4 cases of follicular carcinoma (50%). In papillary carcinoma, 1 case showed 3+ and 1 case showed 2+ positivity with CD56. In follicular carcinoma, 1 out of 4 cases showed 2+ positivity and 1 case showed 1+ positivity with CD56. In follicular adenoma, 5 out of 8 cases (62.5%) showed 3+ positivity with CD56 and 3 cases (37.5%) showed 2+ positive expression.

TABLE 12: GALECTIN-3 EXPRESSION IN MALIGNANT THYROID NEOPLASMS

NEOPLASMS	GALECTIN-3	
	POSITIVE	NEGATIVE
Papillary carcinoma (n=14)	14**(100.0%)**	0(0%)
Papillary carcinoma - Follicular variant (n=4)	3(75.0%)	1(25.0%)
Follicular carcinoma (n=4)	2(50.0%)	2(50.0%)

STATISTICALLY SIGNIFICANT (P<0.05)

CHART 11: GALECTIN-3 EXPRESSION IN MALIGNANT THYROID NEOPLASMS

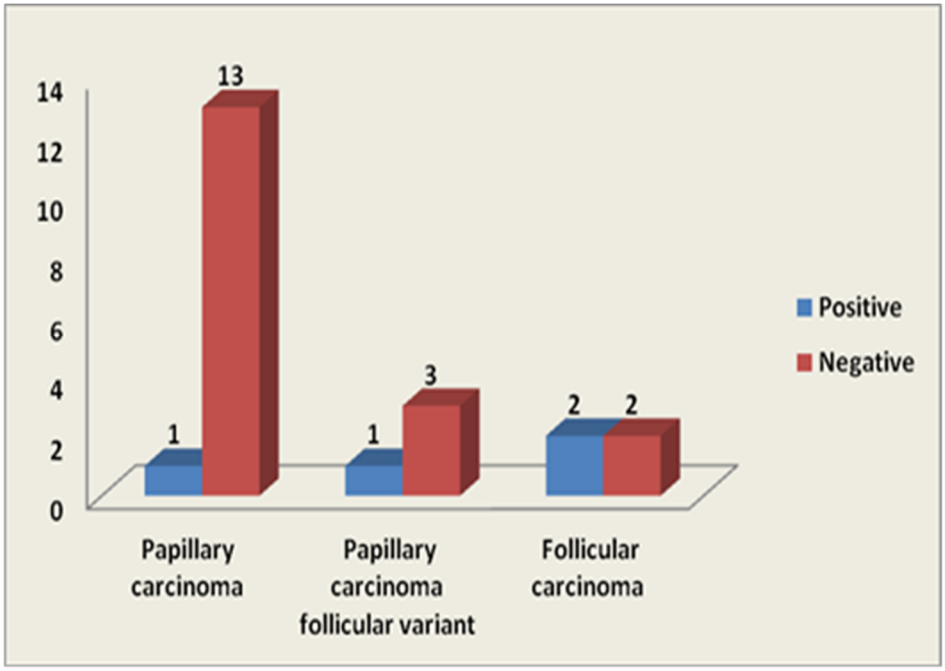


The above diagrams depict the expression of Galectin-3 to be 100% in papillary carcinoma (classical type) with all 14 cases showing positive staining.

TABLE 13: CD56 EXPRESSION IN MALIGNANT THYROID NEOPLASMS

NEOPLASMS	CD56	
	POSITIVE	NEGATIVE
Papillary carcinoma (n=14)	1(7.1%)	13(92.9%)
Papillary carcinoma Follicular variant (n=4)	1(25.0%)	3(75.0%)
Follicular carcinoma (n=4)	2(50.0%)	2(50.0%)

CHART 12: CD56 EXPRESSION IN MALIGNANT THYROID NEOPLASMS

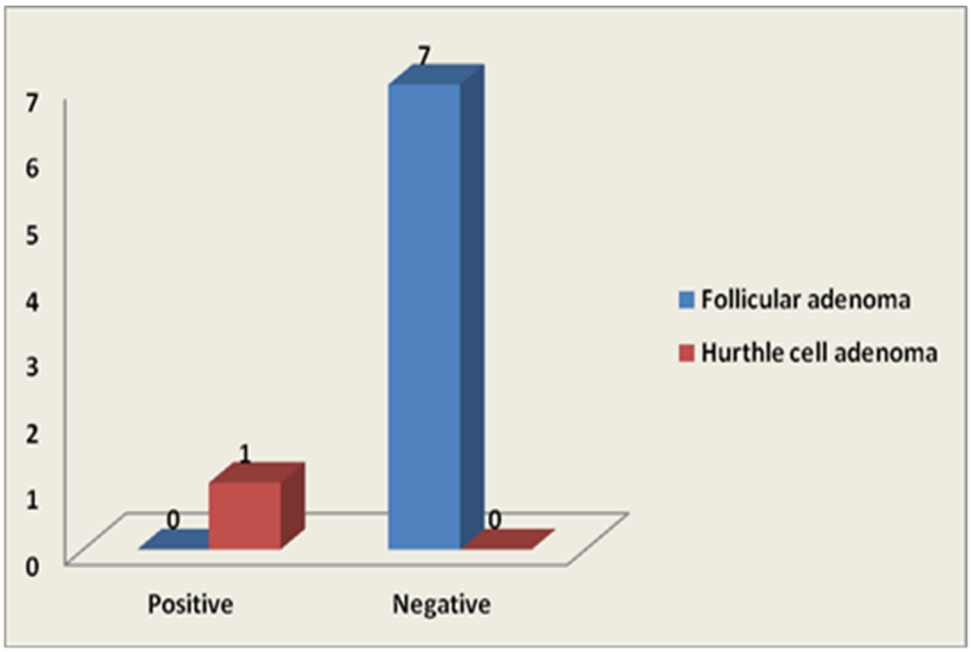


The above diagrams depict the negative expression of CD56 in malignant neoplasms. 13 out of the 14 cases (92.9%) of papillary carcinoma (classical type) showed negativity. Also 3 out of 4 cases (75%) of follicular variant of papillary carcinoma and 2 out of 4 cases of follicular carcinoma showed negative CD56 expression.

TABLE 14: GALECTIN-3 EXPRESSION IN BENIGN THYROID NEOPLASMS

NEOPLASMS	GALECTIN-3	
	POSITIVE	NEGATIVE
Follicular Adenoma (n=7)	0(0.0%)	7(100.0%)
Hurthle cell adenoma (n=1)	1(100.0%)	0(0.0%)

CHART 13: GALECTIN-3 EXPRESSION IN BENIGN THYROID NEOPLASMS

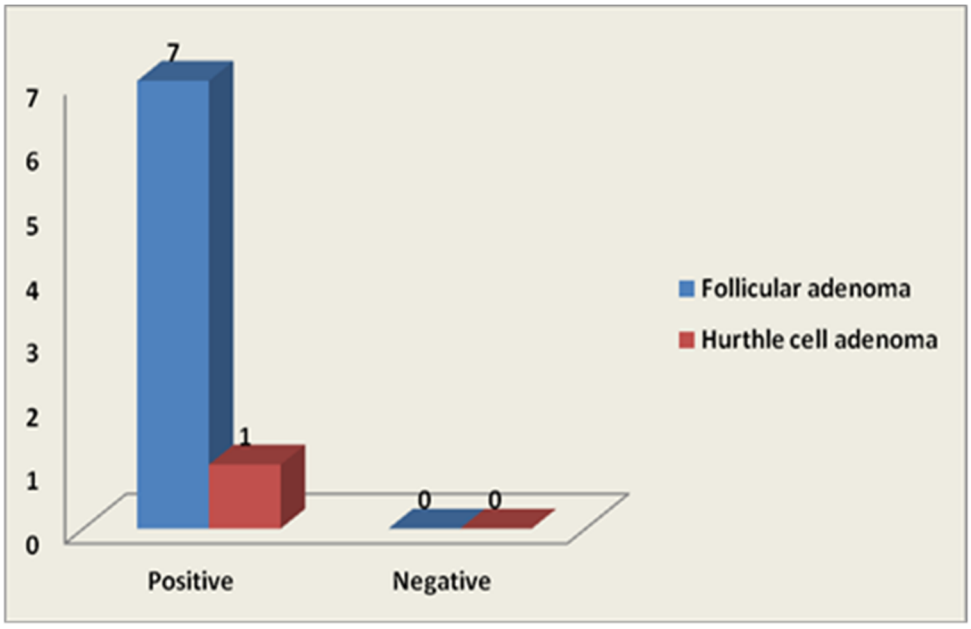


This diagram illustrates the negative expression of Galectin-3 in benign neoplasms, especially follicular adenoma which showed 100% negativity (7 out of 7 cases). One case of Hurthle cell adenoma showed positive expression with Galectin-3.

TABLE 15: CD56 EXPRESSION IN BENIGN THYROID NEOPLASMS

NEOPLASMS	CD56	
	POSITIVE	NEGATIVE
Follicular Adenoma	7(100.0%)	0(0.0%)
Hurthle cell adenoma	1(100.0%)	0(0.0%)

CHART 14: CD56 EXPRESSION IN BENIGN THYROID NEOPLASMS



This diagram illustrates the strong positive expression of CD56 in benign neoplasms. All the benign cases (7 cases of follicular adenoma and 1 case of Hurthle cell adenoma), 100% showed positive staining with CD56, supporting it to be a marker for benignity.

6. COLOUR PLATES

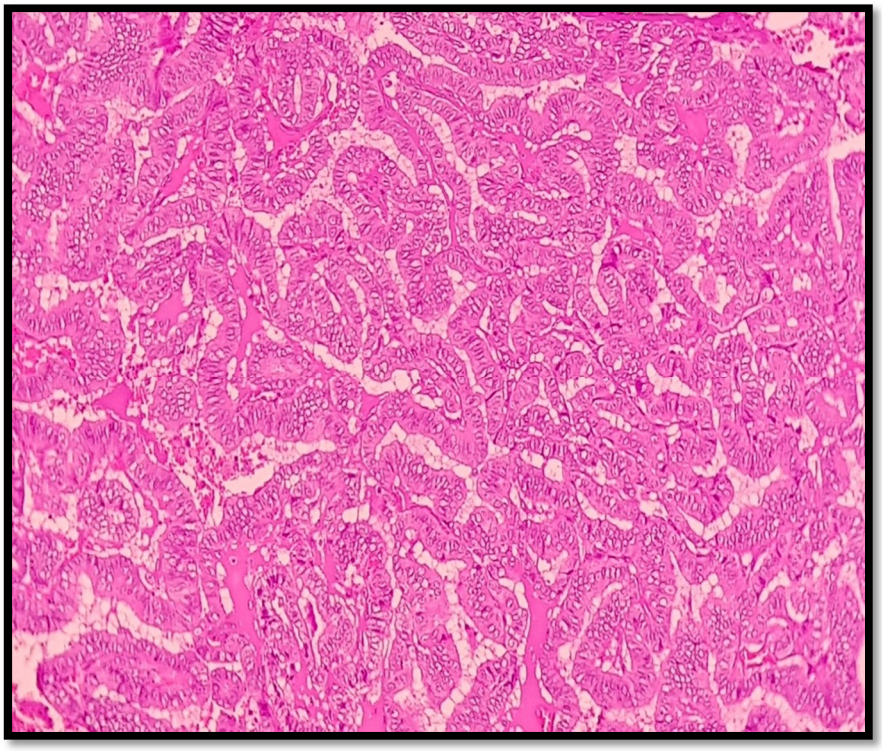


Figure 1 : PAPILLARY THYROID CARCINOMA, H&E STAIN. (100X)

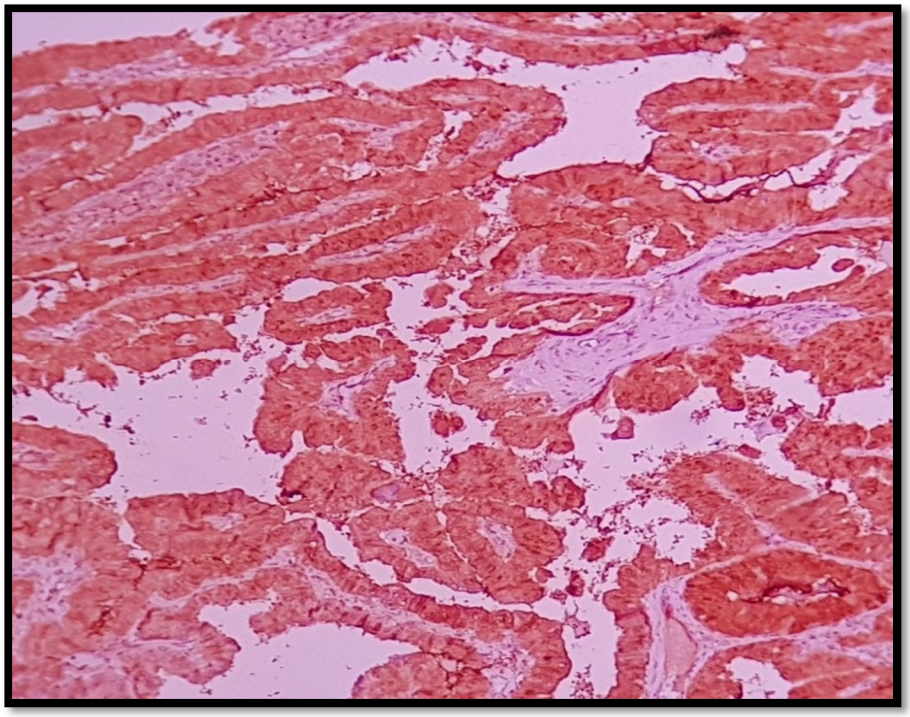


Figure 2 : GALECTIN-3 3+ CYTOPLASMIC POSITIVITY IN PAPILLARY THYROID CARCINOMA. (100X)

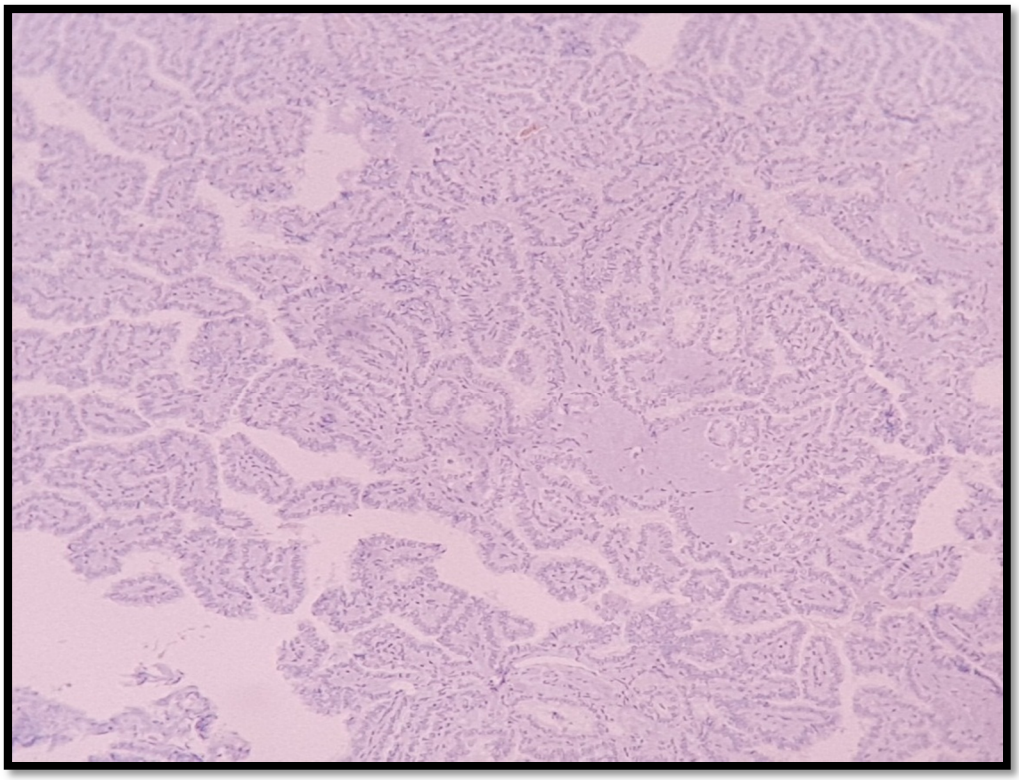


Figure 3 : NEGATIVE MEMBRANOUS EXPRESSION OF CD56 IN PAPILLARY THROID CARCINOMA. (100X)

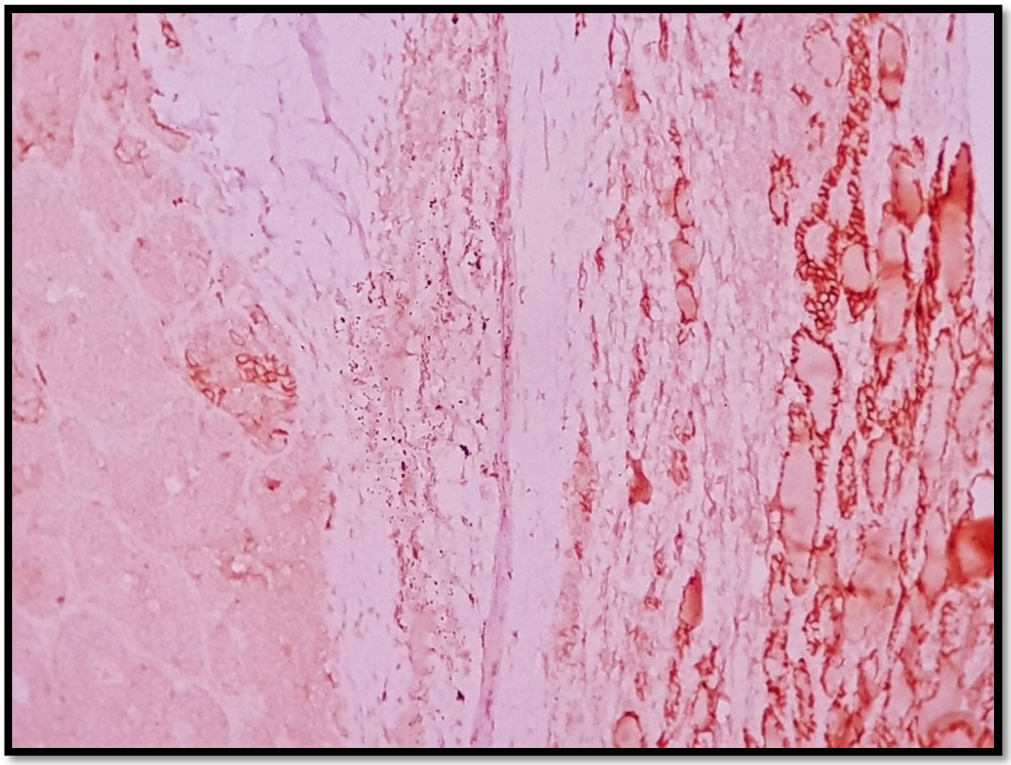


Figure 4 : CD56-NEGATIVE MEMBRANOUS EXPRESSION IN PAPILLARY CARCINOMA (LEFT) WITH POSITIVE MEMBRANOUS EXPRESSION IN ADJACENT NORMAL THYROID PARENCHYMA (RIGHT). (100X)

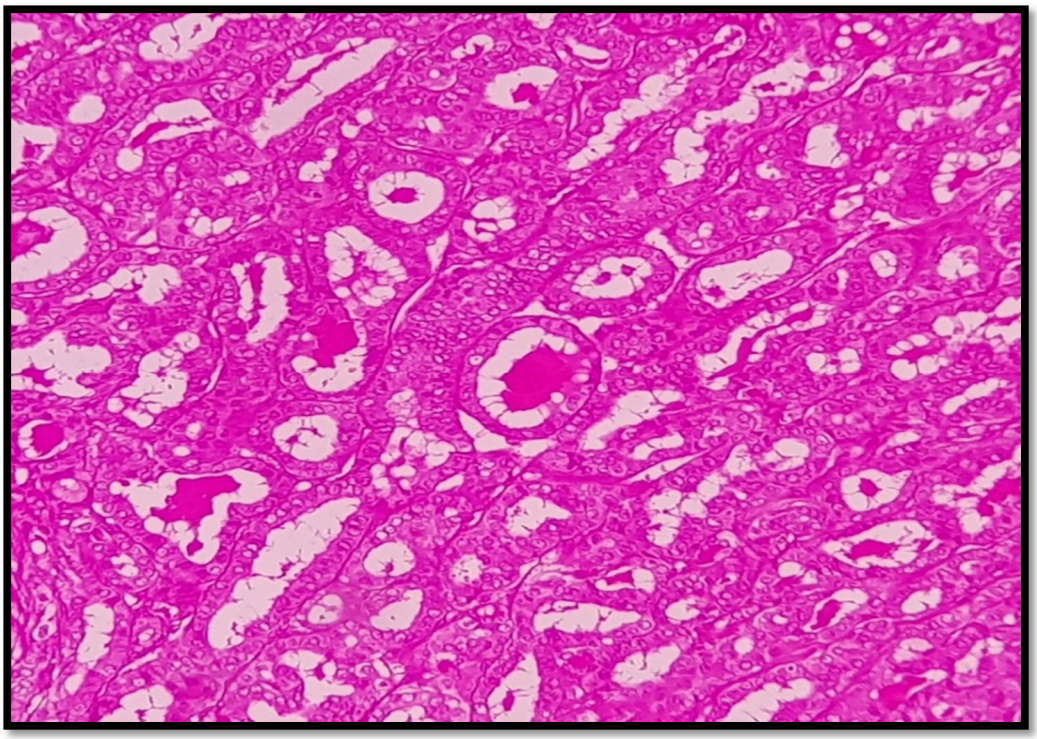


Figure 5 : PAPILLARY THYROID CARCINOMA – FOLLICULAR VARIANT, H&E STAIN. (100X)

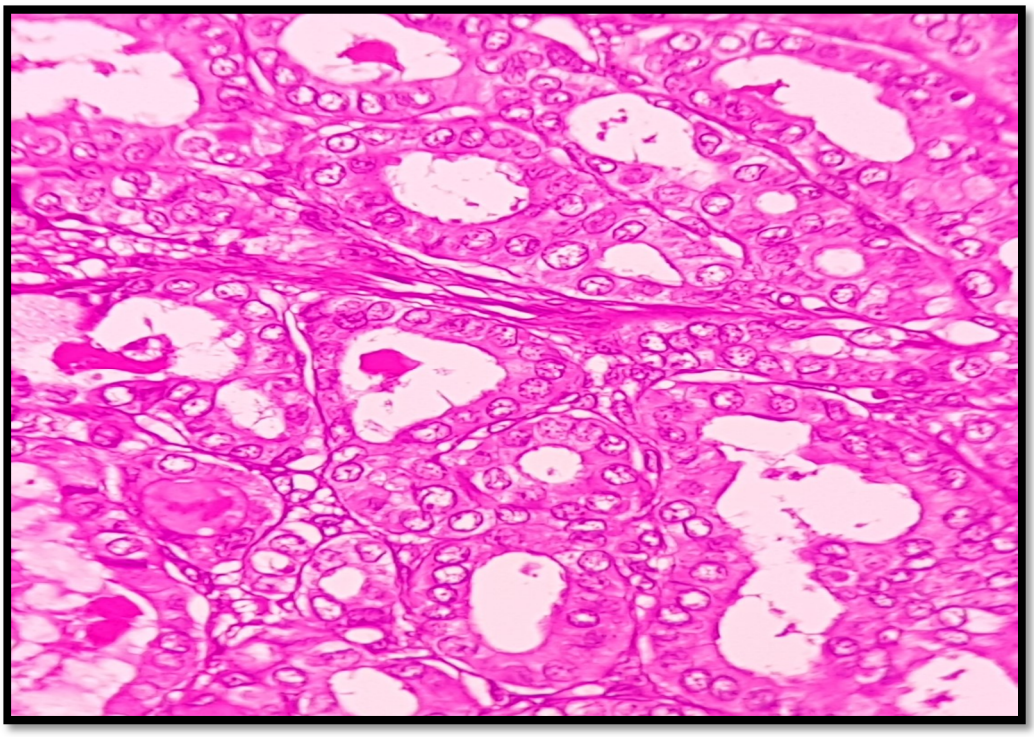


Figure 6 : PAPILLARY THYROID CARCINOMA – FOLLICULAR VARIANT, H&E STAIN. (400X)

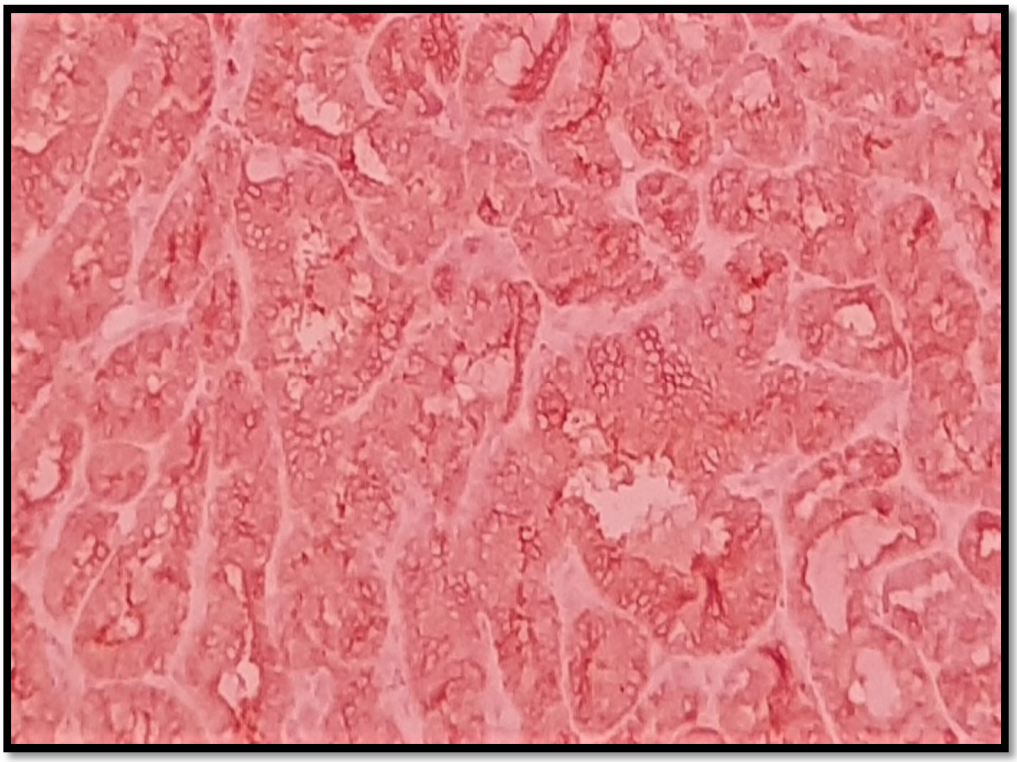


Figure 7 : GALECTIN-3 3+ CYTOPLASMIC POSITIVITY IN PAPILLARY THYROID CARCINOMA- FOLLICULAR VARIANT.(100X)

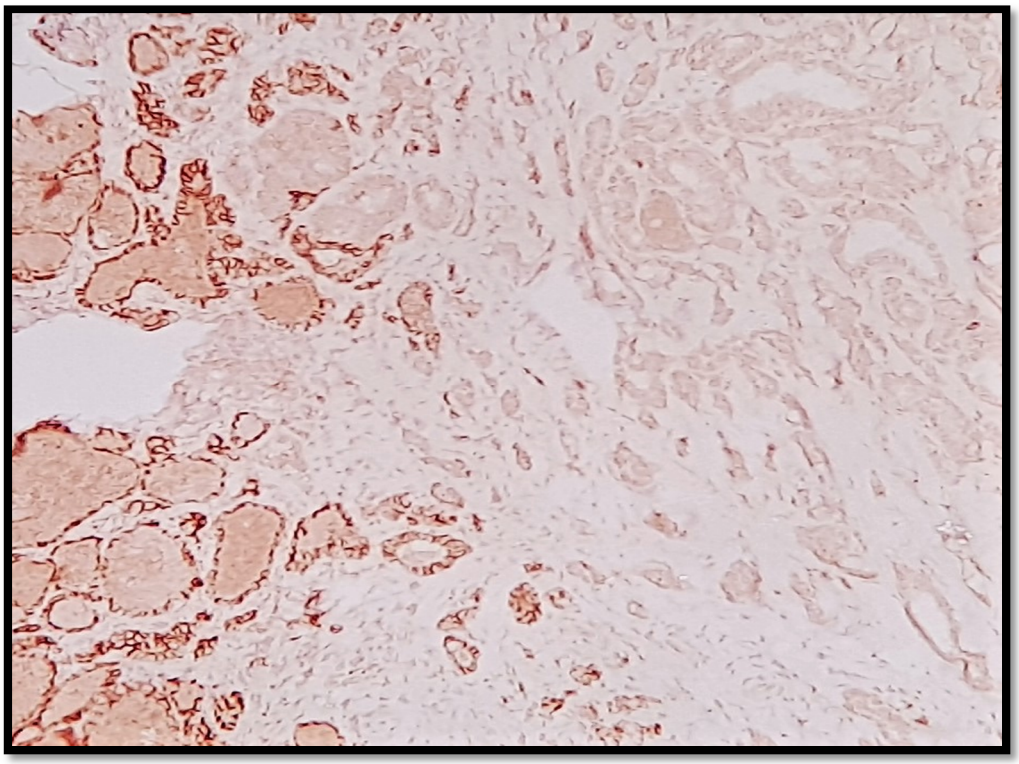


Figure 8 : CD56 MEMBRANOUS NEGATIVITY IN PTC-FV (RIGHT) WITH NORMAL POSITIVE MEMBRANOUS EXPRESSION (LEFT). (100X)

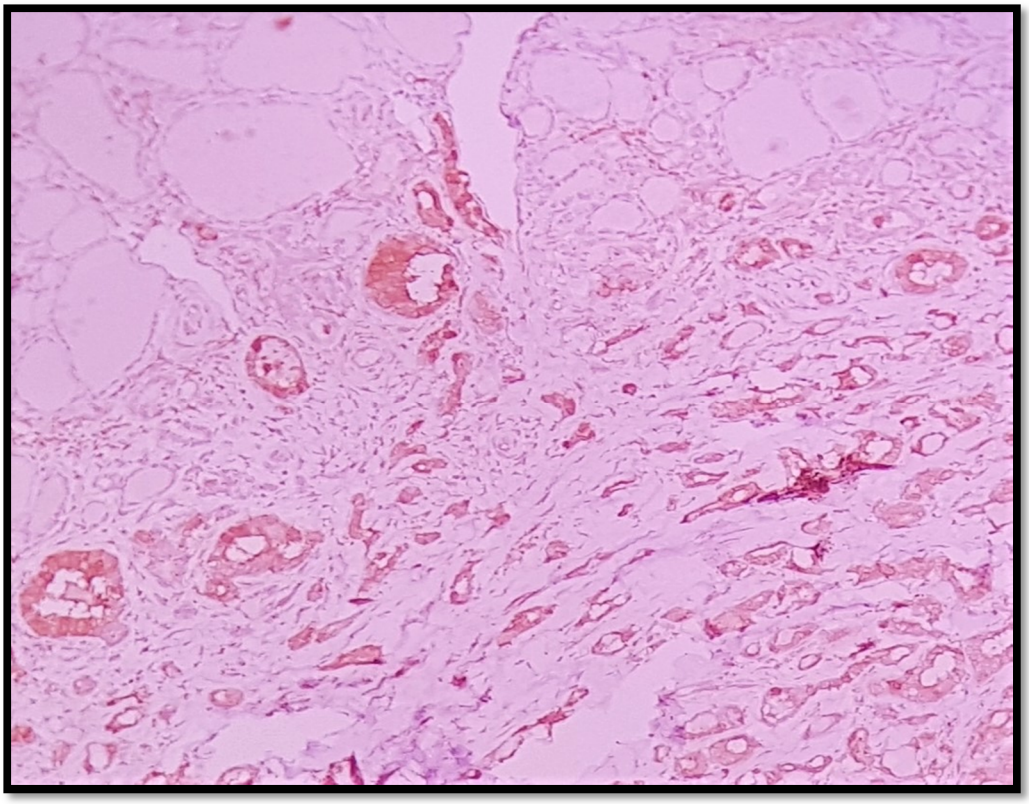


Figure 9 : GALECTIN-3 CYTOPLASMIC EXPRESSION IN PTC-FV, SEEN INFILTRATING ADJACENT THYROID PARENCHYMA ABOVE. (100X)

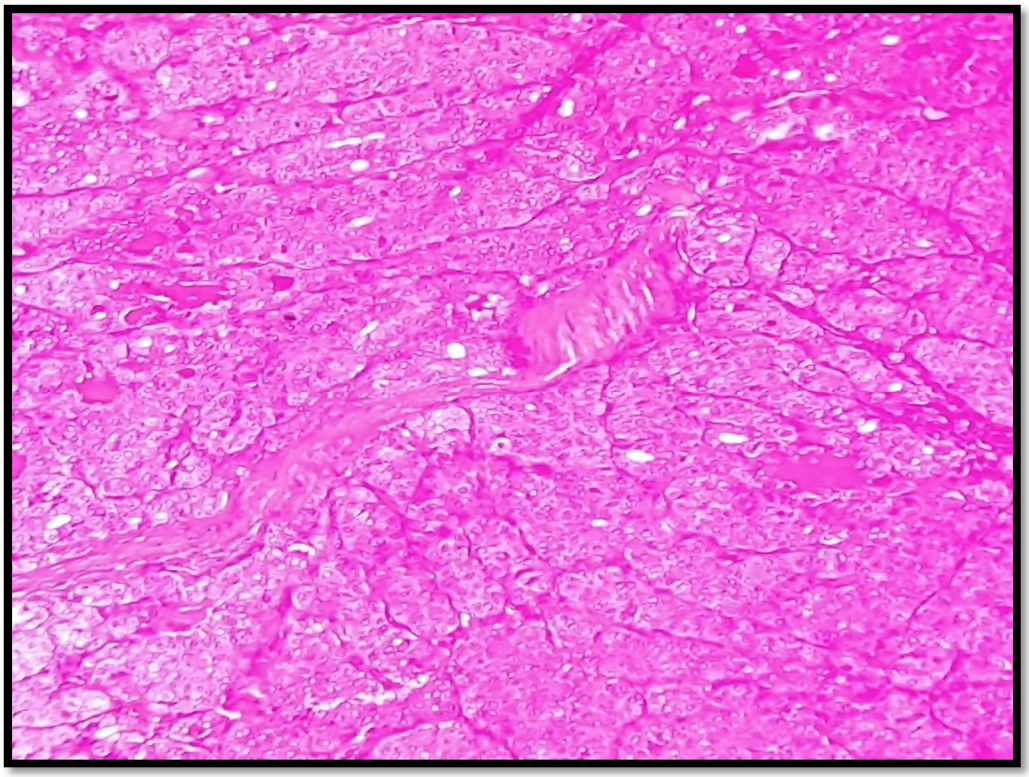


Figure 10: FOLLICULAR CARCINOMA, H&E STAIN. (100X)

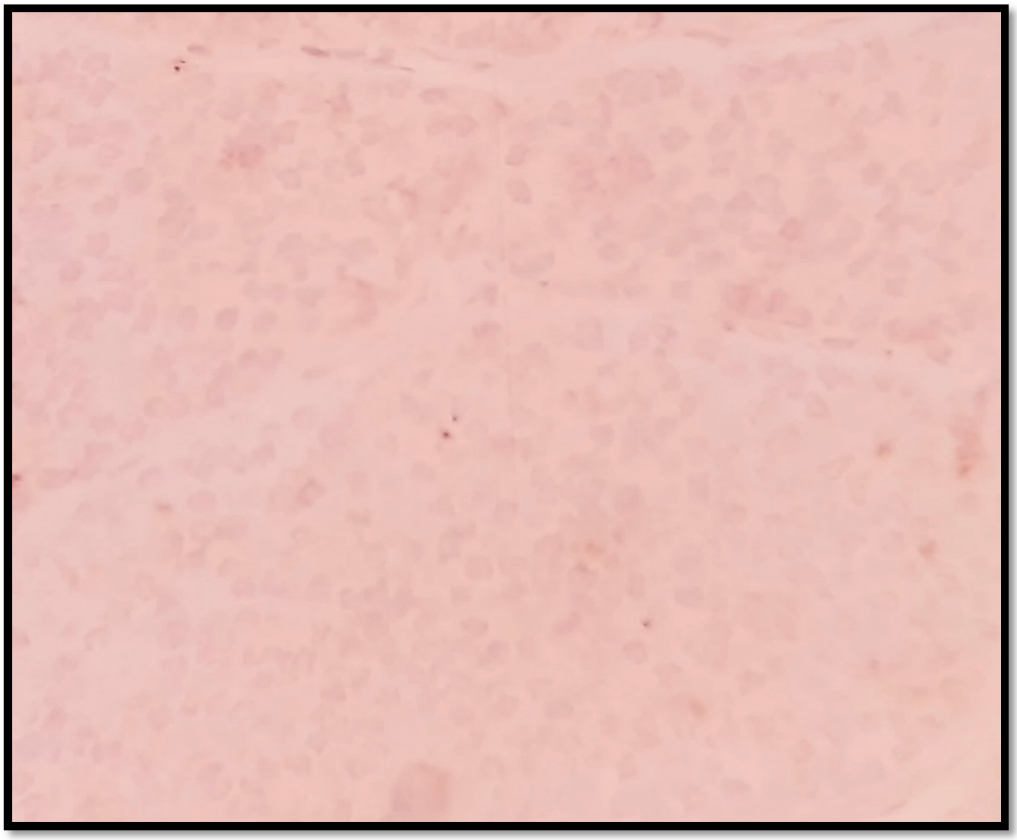


Figure 11: GALECTIN-3 NEGATIVE CYTOPLASMIC EXPRESSION IN FOLLICULAR CARCINOMA.(100X)

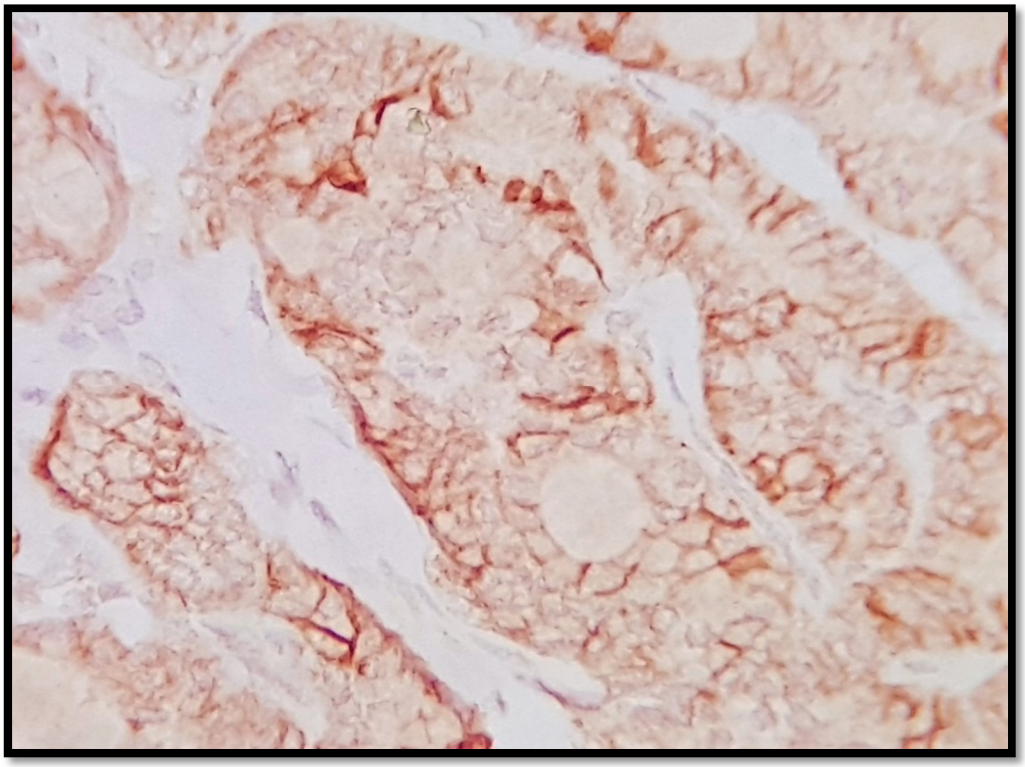


Figure 12 : CD56 POSITIVE EXPRESSION IN FOLLICULAR CARCINOMA.(400X)

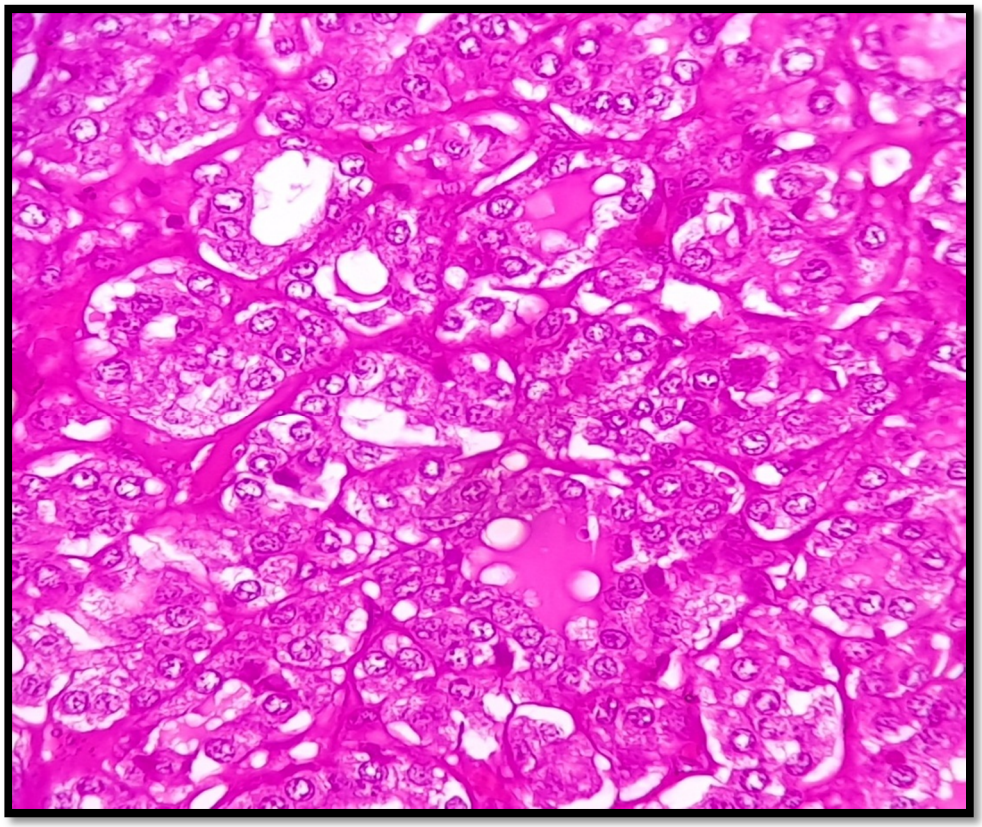


Figure 13 : FOLLICULAR ADENOMA, H&E STAIN.(400X)

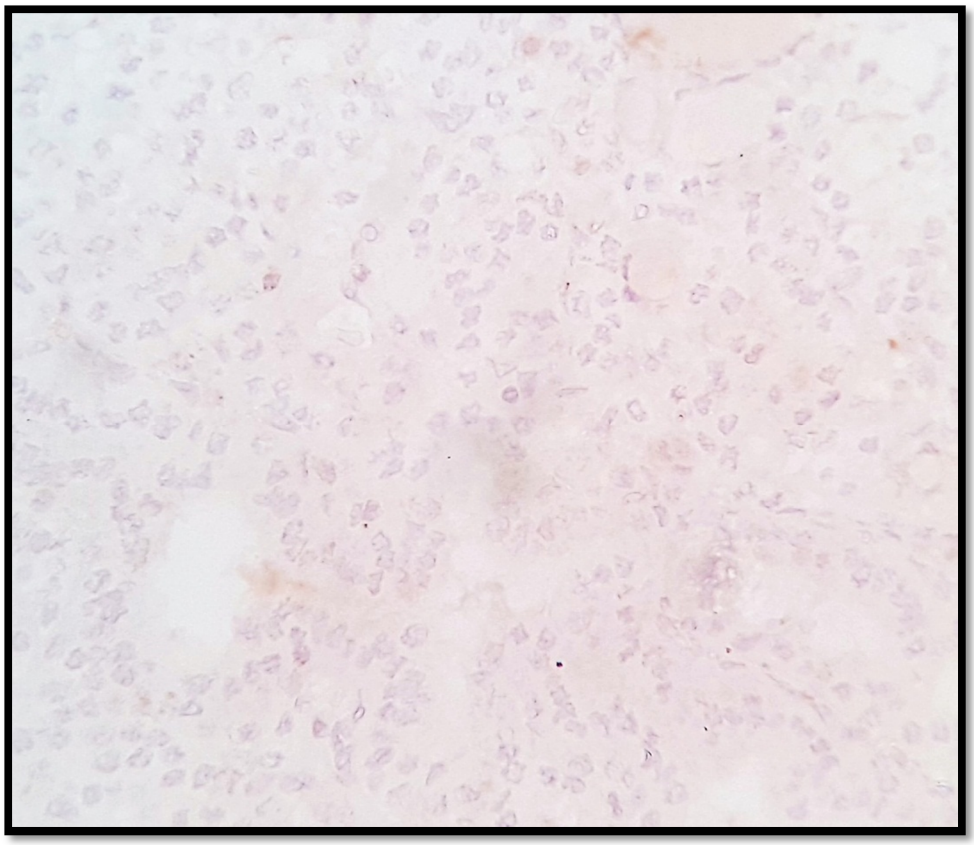


Figure 14 : GALECTIN-3 NEGATIVE CYTOPLASMIC EXPRESSION IN FOLLICULAR ADENOMA. (100X)

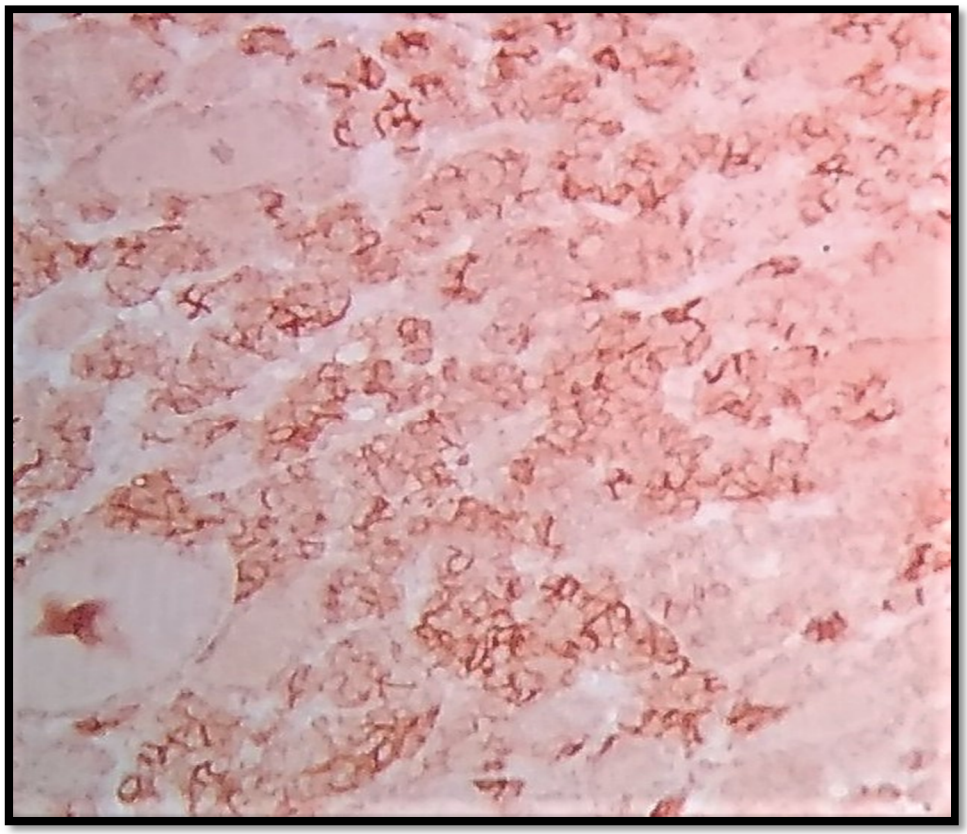


Figure 15: CD56 POSITIVE MEMBRANOUS EXPRESSION IN FOLLICULAR ADENOMA. (100X)

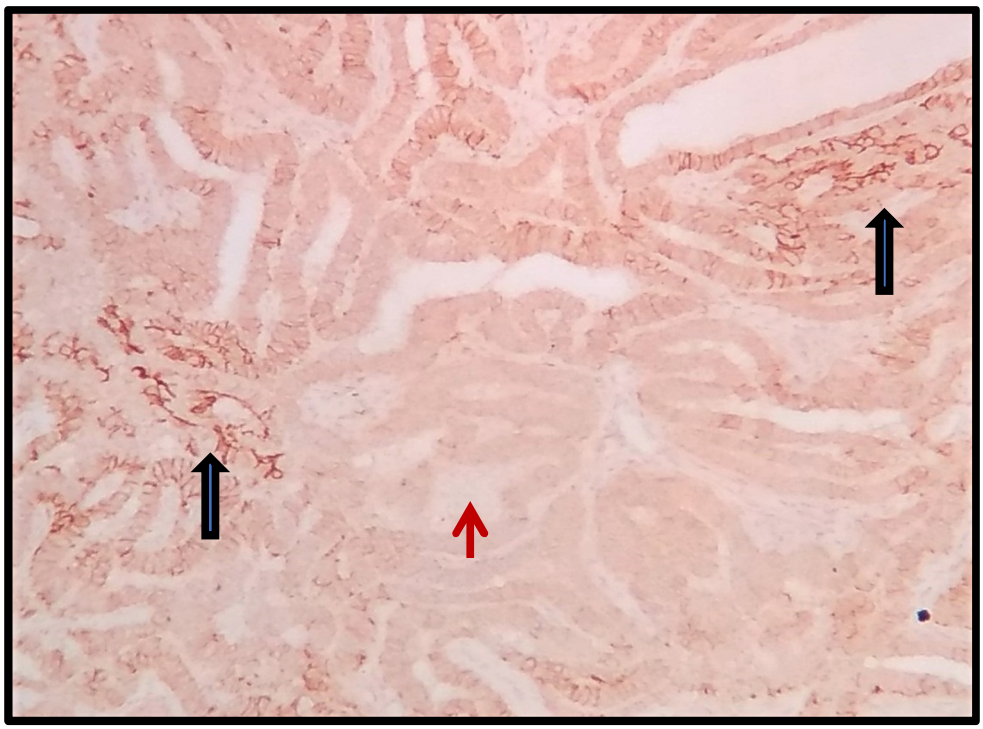


Figure 16 : CD56 MEMBRANE POSITIVITY (>10%) IN ONE CASE OF PAPILLARY CARCINOMA WITH LOSS OF MEMBRANE STAINING (NEARLY 60%) OF CELLS WHICH MAY BE INDICATIVE OF MALIGNANT TRANSFORMATION

7. DISCUSSION

Thyroid tumors are the most common endocrine tumors, originating from follicular epithelial cell differentiation. Papillary carcinoma is the most common type of thyroid carcinoma and its incidence is increasing over the years. This rise in incidence is found to be due to the awareness among the people to seek medical examination even for minor ailments and also due to the advances in medical field, which enables the detection of even impalpable thyroid nodules which may be occult thyroid cancers. Benign tumors should be distinguished from malignant thyroid tumors as it is critical for further treatment and long-term management of the patient. Histopathology always remains the GOLD STANDARD in the part of the diagnosis. But when there are equivocal histological features, there is a chance of underdiagnosis of a given carcinoma or overdiagnosis of a benign neoplasm. For example, the diagnosis of follicular adenoma which is a benign condition and follicular variant of papillary carcinoma which is a malignant condition, is one of the most difficult and controversial issues in thyroid, due to high degree of interobserver variations.[112]

The advent of ancillary test like immunohistochemistry is becoming very useful in discriminating benign from malignant thyroid neoplasms, especially, the follicular patterned thyroid lesions from papillary thyroid carcinoma. In this study, two immunohist ochemical markers - GALECTIN-3 and CD56 have been used to compare and evaluate their use in combination, in distinguishing

benign from malignant thyroid lesions. The malignant neoplasms included are Papillary thyroid carcinoma and Follicular carcinoma. The benign neoplasms included are Follicular adenoma and Hurthle cell adenoma.

PRESENT STUDY:

In the present study, thyroid neoplasms were found to occur predominantly in the age group of 21 to 40 years (16 out of 30 cases), constituting about 53.3%, followed by 41 to 60 years of age (7 out of 30 cases), constituting 23.3%. Papillary thyroid carcinoma was also found to be common among the younger age group between 21 to 40 years (12 out of 30 cases, 40%). Females were more commonly affected (27 out of 30 cases) than males, constituting about 90%. Sumana et al.,[100] had similar finding in their study where 25 out of 50 cases (50%) were found in the age group of 21 to 40 years and females were more commonly affected (37 out of 50 cases), constituting about 74%.

Out of the 30 cases studied, 22 cases (73.3%) were malignant and 8 cases (26.7%) were benign. Malignant cases (n=22) included 18 cases of papillary thyroid carcinoma (60%) (of which 4 were follicular variant of papillary carcinoma) and 4 cases of follicular carcinoma (13.4%). Benign cases (n=8) included 7 cases of follicular adenoma (23.3%) and 1 case of Hurthle cell adenoma (3.3%).

Five cases out of the 18 cases of papillary carcinoma (27.8%) showed metastasis to the regional lymph nodes, which included 4 cases of papillary carcinoma and 1 case of follicular variant of papillary carcinoma. This is much less than the study by Kawachi et al.,[113] where 70 out of 115 cases (60.9%) of papillary carcinoma showed metastasis to the lymph nodes and more than the study by El Demellawy et al.,[114] where only 9 out of 72 cases (12.5%) showed nodal metastasis.

GALECTIN-3 EXPRESSION IN THE PRESENT STUDY:

Galectin-3 belongs to a member of the beta galactosidase binding lectin family and it is involved in the regulation and control of cell growth, neoplastic transformation and metastasis. Higher levels of expression of galectin-3 in malignancy and its absence in benign lesions are in recent days gaining importance in the detection of thyroid malignancy.

In the present study, totally 19 out of 22 malignant cases (86.4%) – both papillary carcinoma and follicular carcinoma, showed positive staining with galectin-3. This included 17 out of 18 cases of papillary carcinoma (94.4%) and 2 out of 4 cases of follicular carcinoma (50%). Strong and diffuse cytoplasmic expression of galectin-3 was present in almost all tumor cells, scoring them for 3+ in 13 out of 18 cases of papillary carcinoma (72.2%), which included 3 cases of follicular variant of papillary carcinoma. This is in accordance with the previous studies by Sumana et al.,[100] where 17 out of 23 cases (73.9%) showed

3+ positivity. In the study by Park et al.,[115] 65 out of 67 cases (97%) and study by Orlandi et al.,[116] 18 out of 18 cases (100%) of papillary thyroid carcinoma showed 3+ positivity with tumor cells. In the present study, 2 out of 4 cases of follicular carcinoma (50%) showed galectin-3 positivity. This finding is similar to the study by Park et al.,[115] and Oestreicher-Kedem et al.,[117] who have also found variable expression of galectin-3 in follicular carcinoma.

Galectin-3 expression was negative in the 7 benign cases of follicular adenoma (100%) in the present study. The one case of Hurthle cell adenoma in this study showed positivity with galectin-3. This is in concordance with the study by Oestreicher-Kedem et al.,[117] where he had similar galectin-3 positivity in Hurthle cell adenoma and he has attributed the positive staining to be due to the presence of Hurthle cell proliferation and not indicative of malignancy. The same case also showed strong and intense membrane positivity with CD56 in our study, favouring it to be an adenoma. Sumana et al.,[100] also reported one case of Hurthle cell adenoma with galectin-3 positive expression.

CD56 EXPRESSION IN THE PRESENT STUDY:

CD56 is a Neural Cell Adhesion Molecule (NCAM) important for cell-cell adhesion. Loss of CD56 expression is found to be associated with malignancy, especially papillary thyroid carcinoma. CD56 decreases tumor invasion by suppressing vascular endothelial growth factor. In the present study, 18 out of the 22 malignant cases (81.82%) showed negative staining with CD56,

of which 16 out of 18 cases (88.9%) were papillary thyroid carcinoma, as supported in the study by El Demellawy et al.,[114] where all cases of papillary thyroid carcinoma lacked CD56 expression, which proved it to be extremely sensitive and specific.

All the 8 benign cases (100%) included in the study (7 cases of follicular adenoma and 1 case of Hurthle cell adenoma) showed diffuse positivity with CD56, supporting it to be a marker for benignity. Similar finding of CD56 positivity in all benign cases (100%) was stated in the studies by El Demellawy et al.,[114] and Golu et al.,.[118] In our study, 2 out of 4 cases of follicular carcinoma were negative for CD56 and the other 2 were positive contributing 50% in each.

Combining the results of galectin-3 and CD56, our study showed that the galectin-3 expression was significantly higher in papillary thyroid carcinoma (94.4%). All the 14 cases of papillary carcinoma (classical type) showed galectin-3 positivity which makes it 100% sensitive. Among the 4 cases of follicular variant of papillary carcinoma, 3 out of 4 cases (75%) showed positivity for galectin-3. Remaining 25% negativity (1 out of 4 cases) in follicular variant of papillary carcinoma is due to the one case showing negative expression. This is due to the small number of cases of follicular variant of papillary carcinoma that were available in our study. There was no significant difference in the expression of galectin-3 between papillary carcinoma (classical type) and its follicular variant.

Absence of CD56 expression is indicative of malignancy, as it was for 18 out of 22 malignant cases (81.8%) in our study. CD56 was negative in 13 out of 14 cases (92.85%) of papillary carcinoma (classical type). 3 out of 4 cases (75%) of follicular variant of papillary carcinoma showed negative CD56 expression.

As to the CD56 positivity found in the 2 cases of papillary carcinoma, one case was classical type of papillary carcinoma showing 3+ CD56 positivity in 60% of the tumor cells. Nearly 40% of the tumor cells showed total loss of membrane staining (Fig 16). Similar positivity with CD56 has been reported in the previous study by El Demellawy et al.,[114] where 6 out of 15 cases (40%) of papillary carcinoma (classical type) showed positive CD56 staining. Park et al.,.[115] in their study reported one case of papillary carcinoma with strong and diffuse CD56 expression. But the presence of metastasis to lymph node together with strong galectin-3 expression in this case confirmed it to be a malignant tumor.

The other case was follicular variant of papillary carcinoma showing 2+ CD56 positivity in 30% of the cells. Nearly, 70% of the tumor cells showed loss of membrane staining. This case also showed galectin-3 negativity. Similarly, Sumana et al.,[100] in their study reported one case of follicular variant of papillary carcinoma with galectin-3 negativity and Golu et al.,[118] in their study

reported 2 cases of follicular variant of papillary carcinoma with diffuse CD56 positivity in more than 50% of the tumor cells.

If the loss of membrane staining in CD56 is taken as a significant finding, then both these cases are showing loss of membrane staining of varying degrees and possibly this may be due to them undergoing malignant transformation. Golu et al.,[118] in their study have mentioned that absence or low expression of CD56 is indicative of malignant transformation.

Galectin-3 expression is found to be statistically significant in malignant thyroid neoplasms ($P<0.05$), especially papillary thyroid carcinoma. CD56 expression is found to be statistically significant in benign thyroid neoplasms ($P<0.05$). This combined panel of markers - galectin-3 and CD56 was very useful in discriminating malignant from benign thyroid neoplasms, especially follicular variant of papillary carcinoma from follicular adenoma.

8. SUMMARY

In our present study, a total number of 30 cases of thyroid neoplasms were examined histopathologically, followed by immunohistochemical staining with two markers, GALECTIN-3 and CD56. Out of the 30 cases, 8 cases were benign tumors, which included 7 cases of follicular adenoma and 1 case of Hurthle cell adenoma. 22 cases were malignant thyroid tumors, which included 18 cases of papillary thyroid carcinoma (14 cases - classical type and 4 cases - follicular variant of papillary carcinoma) and 4 cases of follicular carcinoma.

In this study, thyroid neoplasms were found predominantly in the age group of 21-40 years (53.3%) and females were more commonly affected about 90% of the total 30 cases.

In the present study, there was 100% diffuse and strong positive expression of Galectin-3 in all the 14 cases of papillary carcinoma (classical type), favouring it to be a marker for detection of papillary carcinoma. Among the 4 cases of follicular variant of papillary carcinoma, 3 cases (75%) showed galectin-3 positivity. Absence of CD56 membrane staining was noted in 13 out of the 14 cases (92.9%) of papillary carcinoma (classical type) and also 3 out of 4 cases (75%) of follicular variant of papillary carcinoma and 2 out of 4 cases of follicular carcinoma showed negative CD56 expression.

Galectin-3 in our study showed 100% negativity in follicular adenoma (7 out of 7 cases). All the benign cases (7 cases of follicular adenoma and 1 case of Hurthle cell adenoma) (100%) showed positive staining with CD56, supporting it to be a marker for benignity.

As to the CD56 positivity found in the 2 cases of papillary carcinoma, these cases were showing loss of membrane staining of varying degrees and possibly this may be due to them undergoing malignant transformation. One of them already presented with lymph node metastasis, confirming the malignant nature.

The combination of markers, galectin-3 and CD56 was very useful in distinguishing malignant from benign thyroid neoplasms, especially papillary thyroid carcinoma (follicular variant from follicular adenoma). However, their use in follicular carcinoma could not be well recognised due to limited availability of cases. Hence, further studies in future with more number of cases may be useful for validating their valuableness of expression exclusively in follicular patterned thyroid lesions.

9. CONCLUSION

Galectin-3 is found to be a good marker of malignancy, especially in the diagnosis of papillary thyroid carcinoma, with statistically significant P value < 0.05. CD56 is considered a good negative diagnostic marker for papillary thyroid carcinoma. CD56 is found to be a better marker to indicate the benign nature of the tumor. It's expression is found to be statistically significant (P<0.05) in benign thyroid neoplasms. Absence of CD56 expression is useful for differentiating follicular variant of papillary carcinoma from benign follicular thyroid lesion like follicular adenoma. Hurthle cell adenoma staining positive for Galectin-3 does not indicate malignancy.

We conclude that the combination of Galectin-3 and CD56 is highly valuable in distinguishing malignant and benign thyroid neoplasms, especially in tumors with equivocal morphological features.

Further studies on the loss of membrane positivity in CD56 expression involving larger sample size might unravel possible malignant transformation.

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ANNEXURE – I

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

Name: IP No.:

Age: Ward:

Sex: HPE No.:

Clinical Diagnosis:

History of previous thyroid surgery:

Gross:

- Encapsulated or not.

Microscopic findings:

1. Histopathological diagnosis:
    - Papillary carcinoma.
    - PTC-FV.
    - Follicular carcinoma.
    - Follicular adenoma.

    Presence of capsular or vascular invasion.
2. Immunohistochemistry:

    Galectin-3: Immunohistochemical scoring.

    CD56: Immunohistochemical scoring.

ANNEXURE – II

MASTER CHART

S.NO.	AGE	SEX	IP NO.	CLINICAL DIAGNOSIS	HPE NO.	HPE REPORT	LYMPH NODE METASTA	GALECTIN-3	CD56
1	60	FEMALE	5312	NODULAR GOITRE	378/17	PAPILLARY CARCINOMA ENCAPSULATED	NO	3+	NEGATIVE
2	18	FEMALE	10188	MNG	586/17	PAPILLARY CARCINOMA	NO	2+	NEGATIVE
3	40	FEMALE	18234	PAPILLARY CARCINOMA	1465/18	PAPILLARY CARCINOMA	METASTASIS	3+	3+
4	35	FEMALE	189328	MNG	1626/18	PAPILLARY CARCINOMA	METASTASIS	3+	NEGATIVE
5	31	FEMALE	198813	MNG	2155/18	PAPILLARY CARCINOMA	NO	3+	NEGATIVE
6	38	FEMALE	548	SOLITARY NODULAR GOITRE	75/17	PAPILLARY CARCINOMA ENCAPSULATED	NO	2+	NEGATIVE
7	35	FEMALE	4399	NODULAR GOITRE	265/17	PAPILLARY CARCINOMA	NO	2+	NEGATIVE
8	50	FEMALE	8759	PAPILLARY CARCINOMA	569/17	PAPILLARY CARCINOMA	NO	3+	NEGATIVE
9	37	FEMALE	14299	MNG	745/17	PAPILLARY CARCINOMA ENCAPSULATED	NO	2+	NEGATIVE
10	31	FEMALE	39199	PAPILLARY CARCINOMA	2188/17	PAPILLARY CARCINOMA	METASTASIS	3+	NEGATIVE
11	21	FEMALE	41979	ADENOMATOUS GOITRE	2235/17	PAPILLARY CARCINOMA	METASTASIS	3+	NEGATIVE
12	47	FEMALE	90881	PAPILLARY CARCINOMA	3451/17	PAPILLARY CARCINOMA	NO	3+	NEGATIVE
13	40	FEMALE	206148	COLLOID NODULAR GOITRE	2609/18	PAPILLARY CARCINOMA ENCAPSULATED	NO	3+	NEGATIVE
14	63	MALE	4792	PAPILLARY CARCINOMA	PH459/18	PAPILLARY CARCINOMA	NO	3+	NEGATIVE
15	28	FEMALE	1046	PAPILLARY CARCINOMA	133/18	PAPILLARY CARCINOMA FOLLICULAR VARIANT	METASTASIS	3+	NEGATIVE
16	17	FEMALE	113235	SOLITARY NODULE	3793/17	PAPILLARY CARCINOMA FOLLICULAR VARIANT	NO	3+	NEGATIVE
17	40	FEMALE	181463	NODULAR GOITRE	1285/18	PAPILLARY CARCINOMA FOLLICULAR VARIANT	NO	NEGATIVE	2+
18	40	MALE	184585	NODULAR GOITRE	1409/18	PAPILLARY CARCINOMA FOLLICULAR VARIANT	NO	3+	NEGATIVE
19	67	MALE	127337	FOLLICULAR NEOPLASM	24/18	FOLLICULAR CARCINOMA	NO	NEGATIVE	2+
20	55	FEMALE	177532	SOLITARY NODULE	1179/18	FOLLICULAR CARCINOMA	NO	NEGATIVE	1+
21	65	FEMALE	91026	MNG	2938/17	FOLLICULAR CARCINOMA MINIMALLY INVASIVE	NO	2+	NEGATIVE
22	48	FEMALE	12967	FOLLICULAR NEOPLASM	813/18	FOLLICULAR CARCINOMA	NO	2+	NEGATIVE
23	34	FEMALE	193589	MNG	1924/18	FOLLICULAR ADENOMA	NO	NEGATIVE	3+
24	29	FEMALE	41297	MNG	1916/17	FOLLICULAR ADENOMA	NO	NEGATIVE	3+
25	23	FEMALE	43487	MNG	2078/17	FOLLICULAR ADENOMA	NO	NEGATIVE	2+
26	39	FEMALE	45028	MNG	2111/17	FOLLICULAR ADENOMA	NO	NEGATIVE	3+
27	19	FEMALE	46191	MNG / FOLLICULAR NEOPL	2184/17	FOLLICULAR ADENOMA	NO	NEGATIVE	3+
28	42	FEMALE	47312	MNG	2257/17	FOLLICULAR ADENOMA	NO	NEGATIVE	2+
29	53	FEMALE	83560	NODULAR GOITRE	2858/17	FOLLICULAR ADENOMA	NO	NEGATIVE	2+
30	62	FEMALE	214096	MNG	2909/18	HURTHLE CELL ADENOMA	NO	2+	3+

ANNEXURE – III

ABBREVIATIONS

PTC	**:**	Papillary Thyroid Carcinoma
PTC-FV	**:**	Papillary Thyroid Carcinoma – Follicular Variant
TTF-1	**:**	Thyroid Transcription Factor – 1
HBME-1	**:**	Hector Battifora and MEsothelioma-1
CEA	**:**	Carcino Embryonic Antigen
EMA	**:**	Epithelial Membrane Antigen
MAPK	**:**	Mitogen Activated Protein Kinase
RET	**:**	REarranged during Transfection
a.k.a	**:**	also known as
H & E	**:**	Haematoxylin and Eosin
IHC	**:**	Immuno Histochemistry
DPX	**:**	Dibutylphthalate Polystyrene Xylene
DAB	**:**	3, 3'-DiAmino Benzidine
MNG	**:**	Multi-Nodular Goitre